

THE CONTROL OF DIPHTHERIA

WITH SPECIAL REFERENCE TO THE

(a) Bacteriological.

SCHICK TEST. Schick Test.

PROPHYLACTIC AND CURATIVE.

by

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Introduction.

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I. INTRODUCTION.

II. HISTORICAL OUTLINE. Epidemics occur which cause considerable loss of human life, as well as

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which we have no anti-serum of prophylactic value. It is thought that we might be able to control diphtheria better than the other diseases. But in spite of our increased knowledge of diphtheria as compared with other diseases, we do not seem to have succeeded in checking this disease.

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SECTION I.

Introduction.

The control of diphtheria is still a most perplexing problem, despite the fact that the causal organism is well defined and that we have a specific anti-serum with generally recognised prophylactic and curative powers - yet we find that statistics show a general increased incidence in such widely separated localities as Toronto, London, and Copenhagen, and from time to time epidemics occur which cause considerable loss of human life, as well as serious damage to some of the non-fatal cases, and involves economic loss, since hospital treatment is often expensive.

Measles and Scarlet Fever are diseases whose etiology is as yet unknown or uncertain, and for the former disease we have no anti-serum of proven value. It might be thought that we might be better equipped in controlling diphtheria than the other infectious diseases. But in spite of our increased knowledge of diphtheria as compared with Scarlet Fever and Measles, we do not seem to have made much progress in checking this disease.

The introduction of antitoxin treatment in 1894 has undoubtedly helped to diminish the mor-

talities, and prophylactic injections of antitoxin have reduced the incidence of diphtheria, yet there has been no apparent diminution of seasonal epidemics, and from time to time the disease breaks out and rages amongst the population in spite of our efforts.

We must remember, of course, that the disease occurs in cycles, and there may also be a change in its severity from year to year. There has been a decided change in the clinical picture. The wide extension of the diphtheritic membrane is not seen so frequently now as in the early days; there is not so much "black diphtheria". In the early '80's 43 per cent. of the cases showed extension of the membrane into the respiratory tubes; from 1887 to 1907 this had decreased to less than 20 per cent. From 1907 till 1911 a further decrease to 11 per cent. is noted, and in 1920 only 5 per cent. are recorded (Mullin 1923).

In recent years a further advance has been made in the control of diphtheria on the introduction of the Schick Test, and the active immunization of human beings by means of the injection of toxin-antitoxin mixtures.

It was my privilege to learn the technique

of the Schick Test under the late Dr. Claude B. Ker in 1924, and to assist in Schick-testing patients in the fever wards of the Edinburgh City Hospital, and also to give some slight assistance in a few of the elementary schools of Edinburgh under Dr. William Robertson's diphtheria-prevention scheme.

During the past year (1927) I have been privileged to Schick test some 1000 children attending certain of the elementary schools in the County of Surrey and two Institutions, and to attempt the active immunization of all those persons found to be susceptible, i.e. Schick-positive. I propose to describe this latter work at the end of Section III in the present thesis. A number of years will have to elapse before any opinion can be formed as to the efficacy of the methods of immunization employed against diphtheria. The results reported from great centres of population in the United States of America are in favour of the procedure, and there is reason to expect that our results will prove equally satisfactory.

SECTION II.

Historical Outline.

"How long has Diphtheria been known as a specific disease? Were the old physicians of the school of Hippocrates acquainted with it? The difficulty of answering these questions can be appreciated when we remember that in modern times the clinical features of Diphtheria were just clearly grasped and described by Bretonneau in the early part of last century, and that more than 50 years elapsed before the discovery of the causative organism enabled us to differentiate clearly this disease from those which resemble it". (F. Loeffler, 'The Bacteriology of Diphtheria', Nuttall & Graham-Smith, 1908).

The disease is believed to be one of the oldest of the infectious group of diseases which have prevailed from time to time in epidemic form, but in none of the writings of the ancient Greek physicians, of Hippocrates and his pupils, is one able to locate any reference to the disease. Neither Celsus, Soranus, Galen, nor Caelius Auronius ever made mention of a like condition either as a sporadic or an epidemic malady. On the other hand, the disease appears to have been well known in

Egypt, Syria and Palestine, even in ancient times, as is proved by repeated references to it in the Babylonian Talmud, a work which, though it only appeared in the 5th century, transmitted all the old Jewish traditions (Wunsche Der Babylonische Talmud in Seinen Laggadischen Blitandteilen wortgetren uberlitzt und durch Noten erlantert, 1885), Strack (Einleitung in den Talmud, 1694). In the Berachoth Treatise the following occurs: "There were created 903 kinds of death in the world the severest of all Angina" (according to Strack "Askara"). Angina is like a briar in a bundle of wool which one casts behind one (for it is as difficult for the soul to leave the body as to remove a briar from a bundle of sheep's wool). According to some, it is like a ship's cable in the opening of the throat (for a ship's cable can only be drawn with difficulty through a small hole). The Kuss is "like unto a hair which one draws out of the milk". "Askara", it has been suggested by Strack, is connected with the word "aakhar", to stop up, i.e. designating a death from choking, and therefore should now be translated as diphtheria.

The first accurate description of diphtheria recorded is that of Aretaeus (Mann, Die auf

und gekommenen Schriften Hapadociers Aretaeus aus dem Griechischen übersetzt, 1858) the Cappadocian physician who lived in Rome in the latter part of the first century. This observer gives a faithful picture of the disease, describing the varieties of angina, benign and malignant, and noting the possibility of its extension to the respiratory tract, and the occurrence of death by suffocation, of which he gives a remarkably perfect description, drawing attention even to the characteristic factor. He mentioned the frequency of the malady amongst children and the prevalence in certain countries, Egypt and Syria particularly, whence the names "Syriac and Egyptian ulcer." It is remarkable that he describes no epidemics, a fact which leads to the inference that the disease must have occurred only sporadically and never attained epidemic dimensions. During the 4th century a similar disease became epidemic in Rome and was described by Macrobius. Aetius adds to the description of Aretaeus, a commentary and some particulars which leave no doubt that he had seen the same disease two or three centuries later. Amongst other factors Aetius makes reference to palatal paralysis as one of the sequelae of diphtheria. A pestilence "Esquinancia" (cor-

responding to "Sequenantia" the name subsequently given to Angina Maligna) raged in Europe in the year 580. The same writer (Hirsch) states that Baronius spoke of similar throat pestilences which occurred in Rome, first in 856 and again in 1004. In 1039 an almost parallel visitation took place in Byzantium; the name given in this instance to the disease was "Cynanche". Short (1799), it is said, mentioned a kind of Angina which broke out in England in 1389 and carried off a large number of children. In 1517 the Rhine Country was visited by an epidemic of a disease with "white fungus" in the throat. About the year 1581 a vast pestilence marched over Spain. The disease "Garrotillo" or Morbus Suffocans was undoubtedly true diphtheria and raged in Spain and later in Italy for nearly sixty years. The name of "Garrotillo" was given in Spain, because those who suffered from it perished as if they had been strangled by a cord. The Neapolitans, struck by the most formidable of its symptoms, called it "malè in cannâ", disease of the air-tube or trachea. (Memoirs on Diphtheria, New Sydenham Society, 1859, page 31.)

Many are the descriptions of contemporary writers and numerous are the clinical points they

observed; the white deposits on the pharyngeal surfaces later becoming darker, the laryngeal and occasional nasal involvement, the horrible factor due to gangrene, the suffocative death, are all mentioned. The infectious nature of the disease was suggested by Cortesius who also has given the name "Gaulae Morbus" to the condition. In 1642 the disease "died out". But after a period of quiescence of about seventy years the pestilence again flared up. From Italy and Spain it traversed to France (1730) and thence to Holland. Other contemporary observers include De Fonseca (*Disputationes medicae super ea quae Hippocrates, Galenus Avicenas necnon et alii Graeci, Arabi et Latini de Anginarum naturis speciebus, causis, etc., curiationibus scripsere diversis in locis et circa affectionem hisce temporibus vocatam Carrotillo, 1611*), Villa Real (*De signis, causis, essentia, piognostice et curatione morbi suffocantis, 1611*), Herrera in Spain, and Alaymus (1632) and Cortesius (1625) in Italy. Eventually it reached England and was described minutely by Fothergill, Starr, Huxham and others. Switzerland, Germany, and Sweden also were visited at this period, and simultaneously it was carried to America. In 1771-2 it was epidemic in New York. Samuel Bard

(Trans. Amer. Phil. Soc. Philadelphia 1, 1789) published a brochure entitled "An Enquiry into the Nature, Cause and Cure of the Angina Suffocativa or Sore Throat Distemper, as it is commonly called by the inhabitants of this City and Colony". In it he speaks in such a manner as to substantiate the inference of his belief in the Angina and the Croup being merely varieties of the same group. He appears, however, to have included in his descriptions other of the anginose diseases particularly Scarlet Fever and Measles; for he makes mention of inflamed watery eyes, a livid and bloated countenance, with a few red eruptions here and there upon the face.

Douglass, a contemporary of Bard, appears also to have fallen into the last named error. Caldwell (1816) states that Washington himself contracted the disease at his country seat, Mount Vernon near Alexandria, and that his death was due to this cause.

During the pandemic of the 18th century, the formation of tubercular membrane in the larynx and trachea was first described by Balconius (*Opera Medica Omnia*, Seneva, 1762). Speaking of the death of three children, in his second treatise on epidemics and ephemerides, he describes a disease "affectio orthopnoica" from a dyspnoea causing

death; due to neither a "catarrhus suffocans" he thought, nor a failure of the lungs, but possibly to an affective kindred to the "pulmo reflexus" of Hippocrates. One thing is certain from his description - he regarded the condition from which children died as a new one. Ghisi (Lettere mediche, Cremona 1749) was the first to link the tracheal type with the pharyngeal under the name of "Angina Strepitosa perfida mortalis"; he noted the occurrence of the nasal voice and regurgitation as sequelae to the tracheal form. Tubercular membrane was also described in France by de Nobleville (Histoires et memoires, as above, 1746-7) in Paris. By Starr ('An account of morbus Strangulatorius', Phil. Trans. XLVI, 435, 1752) in England as products of a disease which he termed "morbus strangulatorius". An epidemic in Frankfort in 1758 was written upon by Van Bergen (Nova acta natural curiosorum 11, 157, 1764); he called the disease "morbus truculentus infantum" and described the false membrane thus:-
 Tussi rejecit tubulum membranaceum, qui portis membranae tubulosae per ramos bronchiorum durute morbo generata fuit.

In 1765, Francis Home, a physician of Edinburgh, published a book 'An enquiry into the

Nature, Cause, and Cure of Croup' which he described from 12 cases, as a new disease, known locally on the West Coast as "Chock" or "Stuffing". It was pictured by Home most graphically, and by reason of the symptomatic shrill voice and difficult breathing was termed "Suffocatis Stridula" by him. He noted that it was especially common in children, particularly the very young, and stated that he had neither seen nor heard of a case over 12 years of age. Damp weather, the cold winter months, and propinquity to the sea were, he found, predisposing factors. Two forms were described by him, a simple catarrhal form (benign) and a malignant form showing the occurrence of false membrane in the upper part of the trachea and spreading downwards. The membrane was easily detached from the underlying tissues, if not entirely unattached on account of there being "pus behind it". He particularly mentions the rarity of the new disease, in Edinburgh at least, and states that some may go through a whole life and not see a single case, or, at most only one or two. His treatment consisted in venesection, leeches, blisters to the neck, attention to the bowels, and the inhalation of steam and alcohol vapour. Sudorifics or emetics he found to be of no special utility. The

membrane once having formed, he recommended that its removal be attempted, or, if necessary, that tracheotomy be performed. This treatise which aroused the attention of the whole medical world, actively stimulated the study of the disease, and Home's observations were confirmed elsewhere by numerous writers; some, like Home, only met with odd cases here and there, i.e. sporadic, whilst others described epidemics, the latter writers including Lepecq de la Cloture (*Collection d'observations Sur les maladies et constitutions epidemiques*, Rouen 1778) in the Lower Seine Department and Ramsey (*Med. Trans. Roy. Coll. Phys. Lond. II, 25, 1786 at Chesham*). Cases of the type described by Home were described as occurring together with those of the "angina gangrenosa maligna" type.

Samuel Bard (*Trans. Amer. Phil. Soc. Philad. I, 1789* and Johnstone 'A Treatise on the Malignant Angina, etc.', Worcester 1779) and others hence regarded them all as being of the same nature, and related to one another. The difficulty, however, of actually differentiating these diseases was very great. In fact, it was not until the discovery of the causal agent of Diphtheria was made, that any satisfactory classification could be accom-

plished. The matter was rendered even more difficult when John Millar in 1769 described under the name of "Asmatha acutum" a disease clinically resembling croup in many ways, save in the lack of cough, the rattling rather than whistling respiration and the absence of membrane. Acting upon the impetus given by Home's treatise, the Societe Royale de Medicine de Paris in 1785 offered a prize on the subject "Si la maladie comme en Ecosse et en Suede Soud le nom de croup ou angine membraneuse existe en France?" Numerous were the papers sent in, and the successful one was that of Vieusseux (Memoire sur le croup ou angine tracheale, Geneva 1812) who demonstrated its existence in Geneva and probably in France. The other papers showed its existence in France as general.

From the point of clinical value, however, they added little to knowledge, beyond the fact of diminishing frequency of croup. Interest in the subject of croup was a few years later again stimulated when a nephew of Napoleon I, a son of Louis Napoleon, King of Holland, succumbed to an attack. In June 1807, Napoleon offered another prize for a thesis upon the subject. Royer Collord (Rapport sur les ouvrages due Concours, Paris 1812) adjudicated

cated and awarded the prizes to Albus (*Commentatie de tracheitide infantum vulgo croup vocata*, 1816) and Jurine (*Abhandlung uber den Croup, etc.* - Leipzig, 1816). The essay of the latter was of special interest: "Gangrenous angina, Cynanche maligna, or "angina gangrenosa is a disease which is in a pharyngeal, laryngeal, or tracheal form. In the latter "form it might easily be confused with croup, one of "whose symptoms, namely the pulse membrane, it exhibits, if it did not at the same time possess other "distinguishing characteristics." He then speaks of the differences between gangrenous angina and "the "epidemic gangrenous angina of children", this latter assuming croup-like characters sometimes, which he states are the result of the "putrid influence of "the epidemic." From the study of the writers, and taking into account the "predisposition of children "to it, the rapidity with which membrane forms and "the condition of the spots or ulcers upon the tonsils and pharyngeal region, we are tempted to doubt "the existence of gangrene as a specific disease, in "the majority of these cases, and to regard the disease as modified croup which has merely assumed another form owing to the putrid influence of the "epidemic, and to term it aphthous, putrid or malignant.

"nant croup." He denies the infectious nature of croup, and states that people come to that idea because it occurred in association with epidemics of gangrenous angina, German Measles, Smallpox, Chickenpox and Scarlet Fever. Croup is simply in his idea an adventitious phenomenon and was due to the "checking of the perspiration by the access of cold air."

These essays, however, were directed mainly towards arriving at a routine of suitable treatment and dealt with the question of croup as an inflammatory or non-inflammatory condition. Some held to it being the former and others to the latter, and among the latter partisans Lobstein (*Observations et recherches sur le croup Mem. de la Soc. Med. d'emulation VIII, anne (2) Paris, 1817*) was notorious. He held that croup was not a specific haematogenous condition but a catarrh plus a nervous element. The false membrane was not the crucial factor since the patient often dies despite the removal of the obstructing membrane and the consequent freeing of the respiration. Ten years before Antenreith (*Versuche fur d. Prakt, Neilk. I. 9, 1807*) had expressed similar ideas. He deemed the condition due to a "concentration of irritability towards the

"air passages." Besides application of mercury, he first applied clysters of vinegar in order to drain the morbid material towards the stomach, and later, cutaneous irritants to impel it towards the surface of the body and so divert the concentration of irritability. The pathological products in the throat did not count for much in his view; in fact, because it increased the irritability of the parts, he was inclined to discountenance local treatment. The advisability of tracheotomy he denied. His expressed ideas point to his recognition of the condition as a distinct disease due to a definite pathological substance in the blood, and conferring immunity after recovery. His superstitions as to Constellations, etc., however, so hampered him as to make him unable to declare it infectious. Membranous angina and "Millar's asthma acutum" were identical he states, but he makes no mention of their relation to angina maligna. During the eight years succeeding 1818, epidemics resembling the "Garrotillo" in Spain occurred in Tours, La Ferriere and Chenusson, and it was with the material thus supplied that Bretonneau (*Traite de la Diphtherite*, Paris, 1826) based his observation which he published in his monumental "*Traite de la diphtherite, des inflamma-*

"tions specials due tissues muqueuse et en particulier de la diphtherite ou inflammation pelliculaire connu sous le nom de croup, d'angine maligne, d'angine gangreneuse, etc."

"In the year 1818 the garrison of Bourbon Vendee was transferred to Tours and many fell ill there of a disease characterised by ulcers in the mouth, inflammation of the gums, and breaking of the teeth followed by grey green deposits upon the mucous membranes of the lips and cheeks. From the mouth of the sick persons there emanated a most pestilential odour and the neighbouring lymphatic glands began to swell. The disease was first regarded as Scorbutic gangrene of the mouth, and Bretonneau soon recognised that it could have no connection with scurvy because the persons attacked were otherwise healthy, and also because it assumed all the features of angina maligna, when it attacked the tonsils and throat.

"Bretonneau attributed the striking fact that it primarily attacked the gums to the use of drinking vessels in common. When the garrison was replaced after a time by another section of troops, the disease appeared among the latter in the form of severe angina maligna. Within a few months

"of the introduction of the disease by the soldiers
"of La Vendee to Tours, 60 persons of all ages,
"most, however, children, died from it. Some fell
"ill of severe gangrenous angina, others of typical
"croup. An accurate clinical and pathologico- ana-
"tomical investigation of all these cases led Bret-
"onneau to the conviction that all of them were
"caused by one and the same disease, to which he
"gave the name "diphtherite".

"The production of membrane by the actual
"virus was to him the characteristic mark of the dis-
"ease, because in the severe, apparently gangrenous
"forms he found at the autopsy not the expected gan-
"grene of the mucous surfaces, but a membrane of a
"greyish-green colour, owing to a decomposition of
"the blood mixed with it, lying upon the slightly
"altered tissues.

"This was the source of the pestilential
"odour which had suggested a gangrenous condition.
"The association of malignant angina with typical
"croup, which he had observed in many cases, at
"first led him to think the condition might be due
"to the occurrence of two diseases simultaneously,
"but the observation that adults who were suffering
"from malignant angina could infect children with

"typical croup, and the fact that the deposit on the
 "tonsil and pharynx exhibited the same structure as
 "the membranes of croup, convinced him that croup
 "and malignant angina must be dependent upon the
 "same disease producing factor. He further states
 "very decidedly that scarlatinal angina has nothing
 "in common with diphtheritic angina, because diph-
 "theria membrane is formed upon the mucous membrane
 "and can be detached, whereas in Scarlet Fever the
 "change is in the mucous membrane itself and the de-
 "posit cannot be removed. The keen-sighted observa-
 "tions and deductions of Bretonneau led to the con-
 "clusion that diphtheritis, although occurring under
 "different forms, is a single *eus morbi*, just like
 "Scarlet Fever, Measles and Smallpox." (Loeffler,
 "'History of Diphtheria').

Trousseau (Clinique medical de l'Hotel Dieu
 de Paris, 1828) confirmed and extended Bretonneau's
 fundamental investigations, but differed with certain
 of his deductions and in order to indicate his non
 acquiescence with Bretonneau's idea that the condi-
 tion was a purely local one, he substituted the name
 "diphtherie" for "diphtherite", because he consider-
 ed the specific changes upon the mucous membrane to
 be essentially the local product or manifestation of

a general condition or disease. Bretonneau himself subsequently accepted this new name. He also spoke of it as "Egyptian diphtheria", to commemorate the land of its origin.

A digression is here made to refer briefly to the epidemiology of diphtheria during the 19th century.

During the 19th century the disease spread its tentacles to many parts of the globe. Now quiescent for a period, it would suddenly break out again with added strength. The twenties saw isolated epidemics in Europe. With the forties occurred a comparatively mild visitation involving Europe and America. A period of calm followed for a few years, but the middle of the fifties was marked by a conflagration involving practically the entire world. Commencing in France it marched radially, almost decimating the child population of some districts particularly in Bessarabia. Iceland in '56 encountered the disease for the first time. In two decades or so, hardly a corner of the world remained untouched. In 1866 an epidemic commenced in Peking which carried off thousands of the population. The year 1877 saw Japan attacked for the first time and the disease has remained endemic there since. The

sudden recrudescences of Diphtheria in regions from which it has temporarily disappeared may not entirely be independent of the increased facilities for intercommunication and transit generally.

Trousseau regarded the disease as capable of killing not only mechanically but also by a systematic poisoning. He regards the membrane not as a primary lesion but as a result of infection.

By 1840 the personality of Virchow had begun to loom largely in matters relating to pathological anatomy. The writings of Bretonneau and his pupil Trousseau did not meet with the approval of the German pathologist. Virchow (1844) enunciated his ideas of inflammation on mucous surfaces, classifying the processes as (1) Catarrhal, (2) Croupous, and (3) Diphtheritic. Croupous inflammation he regarded as a superficial inflammation with production of small cells in variable number. Diphtheria he looked upon as a true interstitial process affecting the submucous layers, and properly described as gangrenous.

He opposed the contention that croup and diphtheria were manifestations of disease the products of a common causative factor, and sought to establish the anatomo-pathological distinction.

And so great was the personal power of the pathologist that he soon won over many disciples to his cause. It was not until bacteriology became the hand-maiden of the clinician that these divergent opinions were finally disposed of.

The next forty years were characterised by enormous activity, on the part of the clinician to some extent, but by the laboratory investigator to a far greater degree.

In 1855 Bretonneau publicly deplored the differences between the Virchow School and his own and reasserted his convictions that all forms of the disease were contagious. He quoted the tragic occurrences in the Napoleon family. The illness of the Queen Hortense with a diphtheritic inflammation of the gums, the fatal seizure by laryngeal diphtheria of her eldest son, and the later contraction by the Empress Josephine, her mother, of diphtheritic angina from an extension of which she died of croup a few days later. He spoke of direct communication by way of softened mucous membrane, or abraded skin surfaces and quoted examples from contemporary incidents of a like nature and equally augmentative to his views.

The case of Professor Herpin (quoted by

Jaffe) who contracted Diphtheria of the nose by being coughed upon by a child to whom he was giving attention, his later development of paralysis and ultimate death. Contemporaneously a case occurred at the College de la Fleche in which a child trod with naked feet upon the expectoration of a diphtheritic patient and later developed the specific lesions between the toes. About 1865, a newer theory arose. It was to the effect that croup was set up by the inspiration of acrid irritant substances. Many investigators attempted this means, notably Albers, Jurine and others, but were not successful. Bretonneau, however, produced it by injecting cantharides and olive oil into the trachea; and more striking results still were obtained by Delafond who injected chlorine, corrosive sublimate, sulphuric acid, and ammonia, the last being particularly marked. Bretonneau, however, despite the similarity of cantharides croup in dogs and diphtheria in man, clearly recognised their difference by the marked dissimilarity in their course and unhesitatingly urged the specific differences.

The numerous animal experiments performed in this period cannot be recounted in this short resume. Suffice it to say that the success of this

investigator and the failure of the efforts of that to obtain similar results were mainly the result of the darkness which still hung heavily over the actiology of the malady; only with the advent of the light in the shape of bacteriology was this gloom penetrated and finally eliminated. Principal among the investigators of the later period may be mentioned Billroth, Oertal, Lichtheim, Hueter and Marcuse. The doctrine of the contagium animatum obtained an ascendancy about 1840 by attention being drawn to various fungi found upon the dead bodies of fish, silkworms, etc., and the beliefs that such fungi were the entities causative of death.

Laycock (Med. Times and Gazette XVI. 547, 1859) in Edinburgh in 1859, published his theory that Diphtheria was caused by *Oidium Albicans*. Various observers supported, but others denied, and gave the *Leptothrix Buccalis* the actiological distinction. The fungus or vegetable fons et origo of the disease was the stimulus for many experiments during this decade and the following, principally by Hallin, Oertal, and Nasiloff. Eberth in 1872 in sections from tracheal mucous membrane discovered spore-like bodies in a tough ground work, with many young cells. The mucous epithelium which lay under this formation

was usually present though cloudy and invaded by young cells. Micrococci also he found in the lymph channels, etc., near, but none in the kidneys, spleen, or other organs. Many other investigators supported these discoveries by the results of their experiments. In 1880, Letzerich (Archiv. fur. expr. Pathol. und Pharmakol, XII. 354) cultivated diphtheritic material upon solid medium for the first time, an isinglass medium in special glass containers being used, and which he called "Gallertkammern". The micrococcus diphtheriticus, he states, were in "plasm cells" which he found in the walls of the veins of the heart, and other tissues. Klebs, the year later, did not find these plasm cells in the internal organs, but discovered them in large numbers in the membranes. He made cultures from material obtained by introducing fine capillary tubes into the tonsillar deposit of a child which had died from diphtheria. Thus Klebs and Letzerich were the first to cultivate diphtheritic material upon solid media. To the organism thus found, and later on also found by his pupil Brown, and further studied by him, he gave the name microsporon diphtheriticum. In 1883, at the Congress fur innere Medicin at Weisbaden, Klebs recounted his later re-

searches and described a second organism, a short slender rod, staining with methylene blue, and which he found in the more superficial layers of membrane. The rods contained spores at their ends. This statement was confirmed then by Edlefsen (Verhandl. f. Congr. fur. inn. Med. Weisbaden, 1883). So far, however, Koch's postulates were not all accounted for. In 1884, Loeffler published the results of his experiments which satisfied them. He found two groups of organisms, micrococci in post-scarlatinal diphtheria and in the necrotic material and various bacteria in the surface layers of the thick false membrane with small rods arranged in groups more deeply. Cultivating them in peptone gelatine, and serum containing 25% peptone bouillon, differential examinations and inoculations were possible. Employing the cocci he used mice, field mice, guinea-pigs, dogs and monkeys, and inoculated them in various ways. Certain died of septicaemia, the mice especially, but none with lesions suggesting diphtheria. From this he deduced that the cocci were merely adventitious or only secondary to the rods. Using solidified blood serum containing 25% peptone bouillon, he grew the rods. Inoculation of guinea-pigs gave striking results. Rods, however,

were only found in small numbers at the site of inoculation but none in the blood. Similarly, rabbits showed like results. Owing to the small number of organisms found at the lesion and their absence from the blood and tissues, he inferred that the disease was due to a poison elaborated locally but which got into the blood and was absorbed therefrom. Examining healthy throats, mouths, and teeth, he found the rods in a few cases. This caused him much perturbation and hesitancy to regard the organism as the causal factor. He summed up his extensive researches for and against the actiological significance of the rods (bacilli). With regard to the former, he stated that: (1) they were found in 13 cases of clinical diphtheria with fibrinous exudates; (2) they lay in the oldest parts of the false membranes and occupied a deeper position than the associated bacteria; (3) cultures were highly pathogenic for guinea-pigs and small birds, producing a haemorrhagic exudate and widespread oedema locally, and characteristic internal lesions of a vascular character, such as pleural exudates and congestion of the adrenals; (4) the inner organs, as in man, were free from bacteria; (5) inoculation into the trachea of rabbits, pigeons and fowls deter-

mined the production of typical false membranes, as also did scarification of the cornea of the rabbit, or the opened vagina of the guinea-pig.

Against the actiological significance of the bacilli were the facts: that (1) they were not found in all cases of clinical diphtheria examined; (2) the typical arrangement of the bacilli in human false membranes was not met with in the experimental membranes; (3) inoculation into the uninjured mucosa of the mouth was ineffective; (4) animals which survived showed no paralytic symptoms; and (5) typical virulent bacilli were found in one healthy child.

In 1887, he published further work resulting in the finding of typical lesions post mortem in the stomach. Many observers including Babes, Sørensen, Roux and Yersin, and others, followed the lead thus given. In 1890, Loeffler answered many objectors to his conclusions; an interesting fact mentioned by him was of two guinea-pigs which had survived inoculations but which later had manifested severe paralytic lesions.

He also stated that in 1887-8 he had isolated the poison which he considered to be the disease producing factor by making a glycerine extrac-

tion of the cultures. In 1889, Roux and Yersin prepared the poison - diphtheria toxin - by forcing well-grown broth cultures of *Diphtheria bacilli* through a Chamberland filter. Administered in small doses the toxin present in the broth culture frequently gave rise to paralysis. They held that this constituted the certain proof that Loeffler's bacillus was the virus of diphtheria. The ten years following are replete with incident, investigators in Britain and the Continent and in America performing much work. Max Neisser (*Zeitschr. fur Hyg.* XXIV. 443) in 1897 described the special staining process which bears his name. Particularly into the question of the virility or length of life of the organism was research prosecuted, and in 1894 at a Congress at Budapest, Loeffler enunciated that "Convalescents from diphtheria should not be permitted to resume social intercourse until the complete disappearance of the bacilli had been demonstrated by bacteriological examination", and on the same occasion urged the necessity for disinfection. The immunity and susceptibility of animals also received much attention in this decade, and certain statements, particularly those referring to a diphtheria-like disease amongst birds, particularly fowls, be-

ing identical with diphtheria in the human being were so weighty and apparently well confirmed though actually incorrect that a certain amount of doubt was existent for quite a long period. Up to the present, however, both experiments and observations would indicate that diphtheria is almost entirely spread by human agency. The few years preceding 1890 were also rich in experiments directed towards a fuller understanding of the nature of the toxins and attempts towards their isolation.

Contemporaneously Loeffler and Behring contributed much to the subject of the effects of chemical agents upon the life of the bacilli. At this time also, Behring and Kitasato collaborated in their serum research in connection with Tetanus anti-serum. The year 1891 is marked by the publication by Behring at the International Congress of Hygiene and Demography in London, of his paper in which he makes known his discovery of the antitoxin power of the blood of highly immunised guinea-pigs, that immunity having been attained by way of an artificially produced non-fatal attack of diphtheria. Confirmatory experiments were performed by Fraenkel, Wernicke, Roux and others. From this stage commenced an era of phenomenal changes in the treatment of diphtheria.

In 1893, the preparation of the serum on a large scale was first instituted by Meister, Lucius and Brüning at Hoechst-am-Main.

The year 1894 saw the end of the pre-antitoxin days, by then the serum having become known to most. As time passed, its use, however, became more general and its champions more numerous until now, except in the case of a few misguided individuals, the treatment of diphtheria by antitoxin is the academic procedure. During the years which have elapsed since its institution, many actiological factors have been brought to light, have been tested and, in many cases, have been explained. Similarly, many phenomena concomitant with its clinical employment have been noted and discussed, and theories as to their *raison d'être* expressed. Concurrently the bacteriologist has added to our store of knowledge upon the subject. Larger experience and more detailed knowledge have shown certain dangers, greater or less, which may be encountered in association with serum administration; but these incidents are of such relative infrequency as by no means to be looked upon as detrimental to its value or contraindicative to its exhibition, provided the precautions pertinent to such dangers are taken. To

Emil von Behring history must assign the merit for one of the greatest discoveries ever made in medicine - the discovery of antitoxin.

Nowadays, three things are necessary in order to be certain that a patient is suffering from diphtheria: (1) the presence of the characteristic symptoms, (2) a positive Schick test, and (3) the presence of virulent diphtheria bacilli. The year 1913 saw the introduction of the Schick Test at Vienna, and von Behring in Berlin made the first attempts to immunize human beings by the injection of toxin-antitoxin mixture and, further, Park and Zingher in New York City standardised toxin-antitoxin mixtures, and commenced their great work of immunization among school children.

Matter for debate, to be settled according to the prejudices of the clinician or laboratory worker. The clinical characters are the most important factors in diagnosis, and it cannot be insisted upon too often that no good clinician will ever wait for the results of the bacteriological report, or a Schick test, before administering therapeutic antitoxin to any of his patients whose clinical condition suggests diphtheria. Even if the last two characteristics are known to be negative, antitoxin must not be withheld unless diphtheria can be excluded on clinical grounds. In doubtful sore throat cases a known positive Schick reaction and the presence of virulent diphtheria bacilli would confirm a diagnosis of diphtheria.

In an otherwise similar case a negative

SECTION III.

Diagnosis.

Nowadays, three things are necessary in order to be certain that a patient is suffering from diphtheria: (1) the presence of the characteristic pseudo-membrane; (2) a positive Schick test; and (3) virulent diphtheria bacilli. Should any of these characteristics be absent or unknown, the diagnosis is no longer a certainty but becomes a matter for debate, to be settled according to the prejudices of the clinician or laboratory worker. The clinical characters are the most important factors in diagnosis, and it cannot be insisted upon too often that no good clinician will ever wait for the results of the bacteriological report, or a Schick test, before administering therapeutic antitoxin to any of his patients whose clinical condition suggests diphtheria. Even if the last two characteristics are known to be negative, antitoxin must not be withheld unless diphtheria can be excluded on clinical grounds. In doubtful sore throat cases a known positive Schick reaction and the presence of virulent diphtheria bacilli would confirm a diagnosis of diphtheria.

In an otherwise similar case a negative

Schick reaction would point to some coincident infection in a diphtheria carrier.

Clinical Diagnosis.

An inflammation of a mucous surface or of the skin accompanied by the formation of a definite false membrane, is, in nearly every case, due to the action of the diphtheria bacillus. The presence of albuminuria, especially if the temperature is not high enough to suggest the probability of it being merely febrile, is a very strong point in favour of diphtheria. Age is of some considerable importance as, if we can exclude Scarlet Fever and Thrush in very young children - under seven years of age - any visible patching or speckling of the throat must be regarded with suspicion and treated as diphtheria. The situation of the lesion is very important. Positions especially characteristic of diphtheria are the uvula, the soft palate, and the faucial pillars, all of which are more or less spared by other throat inflammations. Exudation on the uvula should always be treated as diphtheria until it is proved to be due to some other cause e.g. Vincent's angina. If the exudation is limited to one tonsil its unilateral situation should be regarded as suspicious of diphtheria, as the conditions simulating

that disease are usually bilateral. Any speck on the fauces, however much unlike diphtheria, if associated with the slightest suggestion of croupiness or laryngitis, must be treated at once as such.

As to the appearances presented by the exudation, we would expect the lesion to be continuous and not discretely spotted, although diphtheria occasionally starts in the tonsular crypts and may show a follicular appearance for a few hours. While the disease cannot be excluded because the membrane is definitely white or yellow in colour, a pearly grey tint is highly suggestive. In diphtheria, the surrounding mucous membranes are usually much less inflamed than in those conditions in which the exudation resembles it. While there is a margin of congestion round the edges of the membrane, the rest of the throat may be comparatively pale. Marked lesions with little general inflammation should always suggest diphtheria, it being thoroughly understood that much oedema and redness do not necessarily exclude its diagnosis. As regards the behaviour of the suspicious patch itself; if subjected to swabbing, exudation which wipes off readily and without haemorrhage is not likely to be diphtheritic, whereas a patch which is firmly adherent and if detached

leaves a bleeding surface behind it is almost certainly so. By swabbing the throat firmly whatever is detached can be examined and if necessary floated out in water, and its nature can thus be readily determined.

Other points in favour of a diagnosis of diphtheria are absence of pain, and pyrexia moderate in proportion to the lesions or non-existent.

The occurrence of any variety of diphtheritic paralysis in convalescence after a suspected throat is, of course, final. It must not be forgotten that the clinical picture may be complicated by the association of diphtheria with another infection, e.g. diphtheria with concurrent scarlatina, measles, or whooping cough - the former two are not uncommon.

Differential Diagnosis.

(a) Faucial diphtheria.

(i). Follicular Tonsillitis - Onset rapid. Temperature high, 104° Fah. Face flushed. If there is any exudate with this condition it consists solely of mucus, loose or inspissated. There is usually considerable local pain, especially on swallowing. The attack may be ushered in by a slight shivering fit. Diagnosis often extremely difficult, and if there is a history of exposure to diphtheria, anti-

serum should be given without delay.

(ii). Catarrhal inflammation of the Fauces - the inflammation is extensive in this condition but not severe. There is rarely much swelling. There is usually but little definite exudate. The local pain and the constitutional symptoms are slight.

(iii). Quinsy¹ - the asymmetrical swelling of the fauces and the protrusion downwards of the palate on the affected side should make diagnosis easy. Further, in this condition there is often great difficulty in opening the mouth, a disability very seldom noticed in diphtheria. Suppuration results, an event which is rare in diphtheria. The pain is often very severe, high fever is common and sometimes the patient is delirious. The tonsil may be covered with thick inspissated mucus which may simulate a false membrane. It wipes off readily when swabbed.

(iv). Simple ulceration of the Tonsil - this usually follows tonsillitis. The presence of ulceration, unless it be very superficial, negatives diphtheria.

(v). Vincent's Angina - resembles diphtheria more closely than any other disease. It consists of an infective inflammation of the throat accompanied by the formation of a pseudo-membranous slough, which

1. Quinsy = peritonsillar abscess.

ultimately separates, leaving an ulcerated surface. It frequently is seen on the uvula and the margin of the soft palate; and in cases of supposed diphtheria in which bacteriological examination is negative, it is well to search for the spirilla and fusiform rods which are found associated in cases of Vincent's angina. The exudate has a peculiar and offensive odour.

(vi). Septic inflammation of the Fauces - there is severe inflammation, often with extreme swelling and the formation of thick, but not membranous, exudate. Sloughing may take place and deep ulceration result. There is usually prolonged fever and the constitutional symptoms are severe. In one form of this inflammation the lesion consists of very painful inflammatory oedema. The mucous membrane is dusky-red. Occasionally there is also distinct erysipelas of the skin of the face or neck. The constitutional symptoms are of rapid onset and may suddenly assume a most serious aspect. The larynx may be invaded.

(vii). Mumps - A serious error is sometimes made by mistaking diphtheria with great glandular enlargement for mumps. The throat must always be examined.

(viii). Scarlet Fever - the distinction may be diffi-

cult if the patient is not seen until a time when it is possible that the rash of Scarlet Fever may have disappeared. The patches on the throat in Scarlet readily swab off, but in septic cases, ulceration may be mistaken for membrane. The throat itself is usually much more uniformly congested than that of diphtheria, and the congestion of the soft palate is well marked. The condition of the tongue should also give valuable information, and in a doubtful case of early desquamation should be looked for. In the septic cases of Scarlet Fever, most frequently mistaken for diphtheria, the temperature usually remains elevated at a period when in diphtheria it would be normal or subnormal.

(xi). The face also remains flushed throughout, and does not assume the pallor which is well marked in most diphtheria patients a few days from the onset. Bacteriological assistance will often be required, and I must repeat here the fact that the two diseases frequently co-exist.

(ix). Thrush - this condition should always be thought of in young children, and also in adults in the last stages of exhausting diseases such as phthisis. The patches are usually milk-white in colour. They occur in almost any situation and quite frequently on

the palate. There is practically no surrounding inflammation and the pellicle swabs off easily leaving a normal-looking mucous membrane behind it.

Microscopic examination of the pellicle shows characteristic mycelia and spores.

(x). Herpes - occasionally affects the fauces. The vesicles, often broken, and presenting a white base with a red areola, lie quite separate from each other. Sometimes the diagnosis is helped by the presence of crops of herpes on the lips or skin. It must be borne in mind, however, that diphtheria and herpes may be associated together and that the mere presence of vesicles on the lips cannot be taken as contradicting a diagnosis of diphtheria in the fauces.

(xi). Ulcerative Stomatitis - the ulcers often present a sloughing surface which at first sight looks like false membrane, but if they be scraped their true character is at once revealed. In severe cases, a most offensive odour is emitted which is quite different from that of diphtheria. It should be borne in mind that ulcerative stomatitis affects the tongue, lips, gums, and buccal mucous membrane, which are parts very seldom invaded by diphtheria. The constitutional symptoms may be severe.

(xii). Syphilitic Ulceration - the wash-leather base

of a late syphilitic ulcer is not infrequently mistaken for false membrane and therefore for diphtheria, but the presence of ulceration rules out that disease, and other signs of syphilis may be found on examination. The acute syphilitic throat is too red and congested for diphtheria.

(xiii). Tubercular Ulceration - the chronicity of this condition and the presence of tuberculous lesions elsewhere should make its recognition not too difficult.

(xiv). Scalds of pharynx, the action of caustics and curds of milk have caused mistakes.

(b) Laryngeal diphtheria.

Almost any disease in which the symptoms of croup are present may suggest diphtheria; but the most frequently mistaken are simple laryngitis, measles, and retropharyngeal abscess.

Simple laryngitis can be diagnosed with certainty only by bacteriological examination.

Measles often begins with laryngitis but the buccal mucous membrane shows, usually, Koplik's spots, and stomatitis should be looked for; further, such symptoms as sneezing, lachrymation and coughing are usually present.

A similar laryngitis, sometimes actually

obstructive, occasionally occurs in the catarrhal stage of whooping cough.

Retropharyngeal abscess is frequently accompanied by signs of obstruction of the larynx; but inspection and digital search of the fauces will reveal the swelling, which is usually on one side. Fluctuation of the swelling will be found in this condition.

Errors of diagnosis in cases of croup can generally be avoided by systematic examination. The fauces of the patient (usually a child) suffering from croup should be inspected in a good light; if any exudate can be seen, even though it be limited and not distinctly membranous, the case may be regarded as one of diphtheria. If no exudate be seen, the finger should be passed over the pharynx to the larynx, and search made for any swelling, such as a retropharyngeal abscess. A foreign body may also be detected in this way. In adults and older children, the larynx should be inspected with the laryngoscope.

In laryngismus stridulus the symptoms are a sudden spasm of apnoea followed as the cords relax by loud crowing gasps. The patient is often rickety, and is usually under two years of age. Convulsive movements and symptoms of tetany may accompany the paroxysm, and complete relief occurs in the intervals.

Some patients suffering from severe broncho-pneumonia together with much cyanosis and dyspnoea are sent to hospital as diphtheria, particularly if they are at all hoarse. There may be some recession of the lower intercostal spaces which adds to the difficulty, but usually the physical signs are sufficiently well developed to clear up the diagnosis. The diagnosis of papilloma of the larynx can easily be established by laryngoscopy.

Paralysis of the abductor muscles of the larynx may be responsible for a dyspnoea which has been mistaken for diphtheria. In adults this is due to nervous diseases: in children the most likely cause is post-diphtheritic paralysis of which, no doubt, other indications will be present.

Oedema of the glottis is another condition which is chiefly seen in adults and the nature of which may be recognised by a study of the general circumstances of the case.

The pressure of an enlarged thymus gland occasionally causes considerable obstruction or even rapid death from acute dyspnoea, in cases of infants under a year old, but this condition is uncommon.

Vulval diphtheria - The only disease likely to be mistaken for this condition is erysipelas; but in this

there is no false membrane, and the inflamed skin presents a definite margin which is absent in diphtheria. Apart from the presence of false membrane, the other forms of diphtheria can hardly be diagnosed with certainty except by a bacteriological examination; but diagnosis may be assisted by collateral evidence, such as the presence of an undoubted case of the disease in the same family, house or school.

Bacteriological Diagnosis.

The *Bacillus Diphtheriae*, or Klebs-Loeffler *Bacillus*, is the causative organism of diphtheria, and belongs to the generic group known as 'Corynebacterium'. The *Corynebacteria* are non-sporing bacilli, destitute of flagella, and non-motile. They show a tendency to assume clubbed shapes, varying from a mere pointing at one end to a swollen, pear-shaped form. This irregularity in shape is usually associated with a segmented appearance of the protoplasm, in virtue of which the rods appear transversely banded when stained. Further evidence of differentiation within the cell is furnished by the occurrence, in most species, of peculiar granules, showing metachromatic staining, and retaining the stain in the presence of acetic acid. Neither clubbed forms nor metachromatic granules are altogether

confined to this genus, but they are eminently characteristic of it. No member of the genus is acid-fast, but all are in some degree positive to Gram's stain, some strongly so, others including *C. diphtheriae* itself having a tendency to become decolorized with prolonged washing in alcohol.

They are mostly aerobic organisms, but some will grow anaerobically, while others flourish best under microaerophilic conditions.

When sugars are fermented there is never any production of gas. Most of the species fail to liquify gelatin, but a few do so. Apart from *C. diphtheriae*, the members of the genus exhibit little pathogenic power: none of them possess any special resistance against heat or chemical disinfectant agents.

Morphology.

In its ordinary form the diphtheria bacillus appears as a straight or slightly curved rod, measuring some 3 to 5 μ in length, and 0.5 μ or a little more in width. In any culture wide variations are seen in the length of the individual bacilli. The ends may be rounded or distinctly pointed, or one may be rounded and the other pointed. The rod is rarely uniform in diameter throughout its entire

length, one or more swellings being visible in most individuals. When a single such swelling is present at one end only, the bacillus presents a clubbed appearance, the opposite end being usually pointed: when both ends are enlarged the organism has been loosely compared to a dumb-bell. Other examples may present a single central swelling, the ends being either rounded or pointed. Very short bacilli also occur, often somewhat triangular in shape: two such short forms are often in apposition by their bases, and it is believed that they represent the products of a recent fission.

The wide variations in length, combined with the irregular development of the swellings, give rise to the polymorphism with which *C. diphtheriae* is rightly credited. In order to appreciate the polymorphism, it is necessary to study and compare the individual bacilli in a microscopical field: its degree varies with the strain of the bacillus and with the medium upon which it has been grown. But in spite of, or perhaps even because of the polymorphism, the microscopical picture presented by a film of the diphtheria bacillus is one of considerable constancy in its general effect upon the eye of the observer. The rapid bacteriological diagnosis

of diphtheria would indeed be impossible were this not the case. decolorisation more than the remain-

der of the If stained preparations, especially when weak solutions of dye are employed, the bacillus is seen to take the colour very unevenly. Bands of light and dark staining may alternate, or the uneven staining may be quite irregular. The enlarged portions usually take the stain deeply. In addition to these irregularly stained areas, certain granules can usually be made out, particularly when special stains are employed. These granules are called metachromatic, owing to the reddish violet colour assumed by them when stained with methylene blue and certain other dyes. The names polar granules or Neisser's bodies, which are also applied to them, are due respectively to their common position near the ends of the bacilli, and because they can be differentially stained by a process popularized by Max Neisser (1897). Individual

bacilli The granules vary in number from a single one, situated at one end, or more rarely towards the centre of the organism, to 4 or even 5 distributed along the whole length of the bacillus. Owing to their size they may be the cause of the irregularities in the breadth of the bacillus. These bodies

have a great avidity for aniline dyes, and when once stained resist decolorization more than the remainder of the bacillus. Metachromatism is much more conspicuous when the preparation is examined by means of a yellowish artificial light.

When examined by dark ground illumination, refractive granules can be made out in the body of the bacillus, and, in addition, the contents of the bacillus appear to be barred owing to differences in the refractive indices of its contents.

Neither spores nor capsules are ever seen in the unstained preparation, nor can they be demonstrated by staining methods. When specimens of a 20-hour old culture incubated at 37°C. are examined unstained, the bacilli are seen to be non-motile.

Even more striking than the morphology of the individual bacilli is their position relative to one another. *C. diphtheriae* shows the same arrangement as the other members of this group. Individual bacilli may lie singly, but much more frequently they occur in small groups of 2, 3, or more members; these may lie parallel to one another or at various angles so as to resemble the letters V or L, or combinations of these which have been compared to Chinese or, better, cuneiform characters. Chains are

never seen. run will be described as the standard

forms, since this medium is almost universally employed

Morphology as seen in membranes.

When examined in smears from membranes the morphology does not differ from that described above. The organisms are found most abundantly in the more superficial layers, where they usually lie in clumps, being grouped so that they have, not inaptly, been compared to an irregular heap of matches. Sometimes *C. diphtheriae* may be present to the exclusion of practically all other bacteria, but more commonly the surface of the membrane is occupied by dense masses of cocci and bacteria, among which a fair number of *C. diphtheriae* may be seen. These latter, however, occur most frequently just below the surface of the membrane, scattered in loose aggregations or in dense clumps as described above. In this situation they are frequently the only organism present, and are, almost always, more abundant than any other species. It was largely owing to this distribution that Klebs came to the conclusion that they might represent the virus of diphtheria. The deeper layers of the membrane usually contain no bacteria.

Morphology in cultures.

The bacilli obtained from growths on co-

agulated serum will be described as the standard forms, since this medium is almost universally employed for diagnostic purposes.

In general appearance the bacilli taken from a 20-hour old culture incubated at 37°C . are even more polymorphic than those seen in the false membrane. In length they are found to vary from 1 to 12u, or even more. Except in the case of very short individuals, perfectly straight organisms are uncommon, the ordinary form being a slightly curved rod, usually with rounded ends showing, when suitably stained, a metachromatic granule near each end. Bacilli with one or more swellings throughout their length are very common. Clubbed and dumb-bell-shaped bacilli are met with, especially in the longer types. Metachromatic granules are frequently present in the swollen portions, and sometimes in other situations. Occasionally, a central swelling is present containing a metachromatic granule while the ends taper to points. Other forms seen in layer or smaller numbers are:

Short forms staining almost evenly, usually shaped like two cones with their bases in apposition. These organisms usually have a single bar of light staining material situated in the middle, which is

probably the division line. When metachromatic granules are present they are usually small and are situated in the dark staining portions. Such forms are usually considered to be products of recent fission, but occasionally cultures are isolated which consist almost entirely of this type, very closely resembling the pseudo-diphtheria bacillus of Hoffmann. This organism is the 'bacille court' of the French authors (L. Martin 1892). L. Martin states that while such a form undoubtedly exists, it is extremely rare, since he had only isolated one culture in fifteen years.

Streptococcal forms consist of a straight or slightly curved, rather long organism, in the interior of which 4 or 5 somewhat small, regularly placed metachromatic granules may be demonstrated.

Sheath bacilli are usually long, curved organisms containing several metachromatic granules of unequal size and irregularly placed. The body of the bacillus usually takes the stain very faintly.

Barred bacilli are organisms of varying length usually without metachromatic granules. When suitably stained they appear as rods composed of alternating bars of light and dark staining material.

In any culture practically all these forms



may be seen, but the relative number of each form varies in different cultures, and even in those made from the same strain. Indeed, when the different portions of the surface of the same serum slope are examined, very great differences may be detected in the proportion of the different types. 24 and 48 hours

Branching forms. In many cultures, but more especially in those consisting largely of the long variety, branching forms can be found when careful examination is made. The usual type consists of a rather long bacillus with a single branch coming off at an angle - approximately a right angle - to the long axis of the parent stem. The branch almost invariably springs from a thickened portion of the bacterial body which usually stains very deeply, but a metachromatic granule is seldom situated at the junction of the branch with the parent stem. The branch apparently commences as a bud on the side of the bacillus, which grows out to varying length and ultimately may break off, forming an ordinary long form. Occasionally, secondary branches may appear on the first branch and from the parent stem other branches may be developed. metachromatic stain. The body of the

Involution forms. When cultures of almost any strain are incubated for more than 24 hours the so-

called 'involution forms' begin to make their appearance, some media being more favourable for their demonstration than others; for instance, egg medium gives cultures showing a greater number of these forms than Loeffler's coagulated serum. In cultures which have been incubated for between 24 and 48 hours at 37° C. many of the bacilli will be found to have grown distinctly larger, both length and breadth having increased; any irregularity in outline will have become more marked. This increase in size and in irregularity of shape continues, so that if specimens are made from cultures 3 or 4 days old, many bizarre forms may be seen. The bacilli are faintly stained, but scattered through their substance are large deep-staining masses which may be metachromatic. Among the common forms may be mentioned:

- (i). Large curved rods with a fairly regular outline containing large metachromatic granules at each end, and perhaps one or two others, irregular in size, scattered along the middle portion of the bacillus.
- (ii). Club-shaped organisms are common, the enlarged ends being occupied by a large mass of substance taking the metachromatic stain. The body of the bacillus is usually curved and tapers gradually to a point. Other metachromatic granules or dark stain-

ing masses may be distributed irregularly along the body.

(iii). Round or bottle-shaped forms, usually staining deeply, or having a large deep-staining metachromatic mass occupying the major portion of their contents, are also not uncommon.

(iv). Very long forms which contain metachromatic granules more or less regularly placed along the length are also occasionally seen. The last two varieties have been respectively compared to yeasts and portions of mycelium, to which they have a superficial resemblance.

These forms, and others intermediate between them, together with branching individuals, are said to occur more abundantly on medium which is neutral or faintly acid; but for their demonstration a medium capable of giving a good growth of *C. diphtheriae* must be employed, and not one which inhibits the growth, such as is usually employed for demonstrating the involution forms of other groups of bacteria. Since Loeffler's original publication they have almost universally been described as 'involution forms', but it is questionable whether this term should be applied to them if it signifies that they are in a degenerating stage. Several of the more

recent authors hold that they are normal to the life history of the organism, and the same applies to the much rarer branching types. Their constant appearance in cultures grown on media suitable to the growth of *C. diphtheriae* and their absence from cultures made on media containing a high percentage of sodium chloride, used so successfully for demonstrating the true involution forms of *B. pestis*, give support to this view.

Staining of *C. diphtheriae*.

(1). Loeffler's Methylene blue is an extremely useful simple stain:

Methylene blue sat. alcoholic sol., 30 c.cm.

Potassium hydrate sol. (1:10,000), 100 c.cm.

Films - from cultures or a direct smear are made, and stained for three minutes, then wash with water. This preparation does not readily over-stain.

Sections - Stain for five minutes or longer. The application of the alcohol during dehydration is sufficient for differentiation.

(2). Neisser's stain is one of the best differential stains used:

Solutions required:-

(1) Neisser's methylene blue

(2) (a) Cresoidin solution
or (b) Bismarck-brown solution.

(4). (1) Neisser's Methylene Blue:

Methylene blue	1 gram.
dissolved in absolute alcohol	20 c.cm.
and added to glacial acetic acid	50 c.cm.
in distilled water	950 c.cm.

(2) (a) Cresoidin 1 gram.
 Distilled water 300 c.cm.
 (dissolve by gentle heat, and filter).

(b) Bismarck-brown 1 gram.
 Distilled water 500 c.cm.

The films are dried and fixed by heat.

(1) Pour on Neisser's stain and allow to act for one minute.

(2) Wash with water.

(3) Stain with Bismarck-brown or cresoidin for fifteen seconds.

(4) Wash with water and dry.

The protoplasm of the bacillus is brown, while the granules are blue-black in colour.

(3). Pugh's stain gives excellent results.

Pugh's Stain.

Toluidin blue	2 grams.
dissolved in absolute alcohol	20 c.cm.
and added to glacial acetic acid	50 c.cm.
in distilled water	950 c.cm.

The dried film is flooded with stain and heated till steam rises. Stain for one to three minutes. The preparation will not over-stain. Wash with water and dry. This is a very useful and rapid single stain. The protoplasm of the bacilli is light blue and the granules are coloured reddish purple.

(4). Bie's stain for C. diphtheriae.

This is an excellent stain for demonstrating the metachromatic granules:

Methylene blue	.	.	.	2	grams.
Dahlia	.	.	.	0.25	gram.
Absolute alcohol	.	.	.	20	c.cm.
Glacial acetic acid	.	.	.	50	c.cm.
Distilled water	.	.	.	950	c.cm.

The stains are dissolved in the alcohol, which is then added to the mixture of acetic acid and water. In this method the specimen is examined mounted in the stain. A film is made and fixed on a cover-glass. The cover-glass is now mounted on a slide with a drop of the stain, in exactly the same way as a film on a cover-glass is mounted in Canada balsam. The excess of stain is removed by blotting so that the cover-glass does not float about on the slide. Examine the preparation at once under the oil-immersion lens. After a few seconds *C. diphtheriae* are seen with pale blue protoplasm and deep blue granules, while other organisms are feebly stained. The preparation is not permanent, and fades in a short time.

Gram's stain. *C. diphtheriae* retains Gram's stain, but not so intensely as many other micro-organisms.

Culture.- Aerobe; temperature range:- 20°-40° C., optimum - 37.5° C.; although *C. diphtheriae* grows on all the ordinary laboratory media, its growth is best

on serum media and Loeffler's original serum made by mixing serum and glucose broth and coagulating by heat is most commonly employed in the bacteriological diagnosis of diphtheria.

Loeffler's medium.

Cultures made from the false membrane show, even after 6 to 8 hours' incubation at 37° C., minute colonies of *C. diphtheriae*. After 18-24 hours' incubation the colonies have considerably increased in size, and if not too crowded together may measure as much as 1mm. in diameter. As seen by the naked eye the colonies appear as smooth, raised, whitish, translucent, circular spots with a moist shining surface. When abundant and massed together they have a distinct cream or dull yellowish tint, the depth of colour seeming to vary with different samples of serum. When viewed by transmitted light with a lens magnifying 10 to 15 diameters, they are seen to be distinctly granular; the centre, being raised and thickened, appears more opaque than the periphery; the edges are usually quite regular and smooth. In older cultures, the colonies when separated from one another increase in diameter, so that after 4 or 5 days' incubation a single colony may measure 4 or even 5 mm. in diameter. This growth occurs chiefly at the peri-

phery, and thus the colonies have a more flattened appearance, but the central thickening, although not much increased in size, is more apparent. The surface loses its shining aspect and may become irregular owing to the formation of fissures and ridges, while the edges are indented. When the colonies are crowded together they tend to coalesce into a sheet of growth with a smooth, dull, yellowish surface and a thinner and somewhat irregular edge. This appearance, however, is only observed in primary cultures when the medium is accurately prepared.

When the colonies are magnified about 20 or 25 diameters, the appearance differs according to the age of the culture. After 20 hours' incubation at 37° C., the central portion is seen to be distinctly thickened and heaped up; outside, the peripheral portion gradually slopes to the edge, which is quite sharply defined and regular. The most notable feature, however, is the coarsely granular structure, which gives to the colony a whitish, opaque appearance, a feature which often enables the observer to identify a colony of *C. diphtheriae* when surrounded by those of other organisms. After 2 or 3 days' incubation the central portion appears even more thickened, and rises sharply from the surrounding

peripheral part, which is proportionally larger than in the younger colonies; the edge becomes less regular, and notches and fissures may appear, but these latter usually extend only for a short distance into the colony. Later, folds and ridges may be seen in the peripheral portion. The structures becomes even more granular, and the colony, in consequence, is more easily distinguished from those of other organisms.

In streak cultures a continuous band of growth develops along the track of the needle, broader below than above, yellowish in colour, thicker in the centre than at the edge, which is usually somewhat irregular. The surface in very young cultures is moist and shining, but in the older ones drier and dull.

Ordinary agar - On good nutrient agar, most strains of *C. diphtheriae* grow well, but not so rapidly as on Loeffler's serum. After 24 hours' incubation the colonies developing on an agar slope are still very small, especially when the culture is made from a false membrane, but after several generations on artificial media growth may become more rapid and abundant. Two types of colony are usually to be seen, a small and a large; the first type is much the more

numerous. (i). The small colony is greyish white, somewhat translucent, with a raised, rounded surface and usually a smooth circular margin; occasionally, however, the latter is irregular and indented. The centre is thickened and, in consequence, appears more opaque, and the structure is distinctly but finely granular. (ii). The larger colony is whiter, smoother, very finely granular, and almost always has a smooth, circular outline and a large central thickened portion.

Subcultures made from either of these colonies give rise to growths showing both types of colony, and no difference can be demonstrated in the morphology, pathogenicity, or other characteristics of the bacilli forming them. In primary agar cultures, however crowded the colonies may be, they always remain separate from one another; after repeated cultivation the colonies may coalesce to form a sheet of growth. In stab cultures growth takes place along the whole length of the needle track, appearing as a greyish white line or series of dots. The surface growth consists of a thickened central portion having a smooth, rounded surface with a thinner peripheral border, the margin of which is usually irregular. In shake cultures growth appears not

only on the surface but throughout the whole mass of the agar, showing that the bacillus is a facultative anaerobe.

Streak cultures show a whitish band of growth along the needle track, broadest at the bottom of the tube and tapering towards the top, where it often breaks up into separate colonies. The band of growth is thickest in the centre, and the margins are usually irregular. The structure is finely granular.

The morphology of the bacilli grown on agar differs in several respects from that observed in cultures on serum. In general the bacilli are more regular in shape, metachromatic granules are developed later or not at all; short forms are commoner and the so-called involution forms are not so well developed. The bacilli, however, have the same arrangement, and most resemble the intermediate forms of those cultivated on serum.

Glycerine agar containing not more than 3 per cent. of glycerine, is superior to ordinary agar as a medium for *C. diphtheriae*. The colonies are similar to those grown on ordinary agar, but they are slightly larger in size. The morphology of the bacilli also resembles that of organisms cultivated on agar.

Glucose agar containing 1 per cent, glucose is also superior to ordinary agar as a medium for *C. diphtheriae*; the growth in all the forms of culture resembles that on ordinary agar, except in the case of the shake culture. In this case a curious phenomenon is constantly observed, namely that when a fairly copious shake culture is made, the colonies develop abundantly in the depths of the medium, but little or no growth occurs on the surface; in fact the culture has a superficial resemblance to that of an anaerobic organism. A possible explanation is that the organisms growing in the depths of the glucose agar do not break down the sugar and consequently produce no acid, while those on or near the surface attack the sugar vigorously and produce sufficient acid to arrest development of the colony.

Gelatin cultures - These cultures on gelatine, whether in streak, stab, or shake cultures, are very similar to the corresponding cultures on agar, but the growth in every case is very much slower owing to the lower temperature. The colonies continue to increase in size for several weeks when the culture is kept at room temperature. Even when incubated at 22° C. the development is slow. The gelatine is never liquified by *C. diphtheriae*.

Broth Cultures - The growth of *C. diphtheriae* in ordinary nutrient broth is rapid and abundant, and usually has a somewhat characteristic appearance. Although for the first few hours of growth the broth may be evenly clouded, the bulk of it rapidly clears, owing to the bacilli collecting into clumps and sinking to the bottom of the flask; at the same time a film of growth spreads over the surface, its formation commencing at the junction of the surface with the side of the flask. This surface film tends to break up at the slightest movement, and the granules composing it sink to the bottom. The formation of the clumps is due to the mode of division of the bacillus, which takes place by the bending and breaking of the parent organism into two, but the division is not so complete at one side and the bacilli take an L, V, or cuneiform arrangement, and thus one finds the coherence of the bacilli after the division has taken place. acids produced in the course of metabolism.

The typical broth culture after 3-5 days' incubation is as follows: the bulk of the broth is brilliantly clear, but there is a film of growth over the whole surface, which on close inspection is seen to be made up of small rounded clumps. At the junction of the vessel wall and the surface of the broth

this film is considerably thickened, and is quite continuous. Sticking to the side of the flask are a variable number of clumps, and the bottom is usually covered with a thick deposit of these rounded masses of bacteria. The slightest agitation causes the whole surface film to break up and sink rapidly to the bottom. The granules of growth, when seen singly, have a greyish-white colour, but in mass may have a slight brownish-yellow tinge. During the growth of the bacillus the reaction of the broth alters. This change was first observed by Roux and Yersin (1888) and has been minutely examined by Spronck (1895), Theobald Smith (1897) and Madsen (1897). The usual sequence of changes is as follows: if the broth is alkaline to commence with, the reaction gradually approaches neutrality, or may in some cases become slightly acid; later still, it again becomes alkaline. The change of reaction is due to organic acids produced in the course of metabolism, and these are later transformed to carbonates producing the alkaline reaction. This is the usual course of events, but if glucose has been added to the broth in amounts of 0.2 per cent. or more, the broth becomes so acid that the growth of the bacilli is inhibited, and, therefore, the secondary alkaline

phase is never produced.

During the growth of the bacillus in broth the toxin is produced, and the question of the reaction has important bearings on its production.

Glucose broth - In broth containing 1 per cent. of glucose, growth is at first very rapid, but the medium soon becomes intensely acid, the bacilli settle down to the bottom and sides of the vessel in an adherent mass, and growth ceases.

Milk - Rapid growth takes place in milk (Zarniko). The milk becomes acid during the growth of the bacillus, but most authors state that no clotting is observed. The preliminary reaction of the milk and the amount of acid produced may be factors which would account for some or all of the divergent opinions concerning the growth of the bacillus in this medium. Several special selective media have been devised to facilitate the isolation of the *C. diphtheriae*, and the best of these is Douglas' tellurium-trypsin agar. The reagents required for this medium are: (a) Nutrient agar; (b) solution of potassium tellurite 1 per cent.; (c) sterile trypsinized serum.

As this medium is perfectly transparent, and as colonies of the *C. diphtheriae* and some organ-

isms of the same group can be recognised with considerable certainty by inspection, it is advantageous to implant the surface somewhat sparsely so that individual colonies may have space to develop, and may easily be picked off for isolation or microscopical examination. After 18 to 24 hours' incubation, if *C. diphtheriae* or certain other organisms of this group be present, colonies having the following characters will be apparent: in size they vary from about 0.75 to 1 mm. or more in diameter; in shape they resemble a somewhat flattened hemisphere with regular outline; in structure they are remarkably granular - a feature best made out with the aid of a hand lens; in colour, which is the most striking character, the centre is a dusky grey-black, while the peripheral portions are a somewhat opalescent greyish-white, the whiteness being accentuated by the granular structure. Colonies of Hoffmann's bacillus have almost identical characters; the blackish colour of the centre is, however, usually less intense. *C. xerosis* and some other members of the diphtheroid group give colonies which are similar in every way to those of *C. diphtheriae*. Colonies of streptococci, which are usually the most abundant associated organisms, are very small, flat, and so

translucent as to be visible only on close inspection. They have no trace of black colour at this stage of development. Only one organism has been met with which at this stage of growth gives a colony in any way comparing in size and appearance with those of *C. diphtheriae*. This is a *Staphylococcus* which occurs in a small percentage of throat swabs. The colonies, however, differ from those of the diphtheroid group mentioned above in that the black colour is much more intense and more diffused throughout the colony. The granular structure which is such a marked feature of the colonies of *C. diphtheriae* is replaced by a homogeneous smoothness - in fact, the colony resembles very closely a spot of wet, black paint.

After incubation for 48 hours, the colonies of *C. diphtheriae* have increased in size so that they measure 2 or more millimetres in diameter. The black colour is much accentuated, and has spread throughout the whole colony. The central portion is usually somewhat thickened, the edges are smooth and regular; the general appearance at this and later stages of incubation may be compared to a flattened drop of partially dried paint. Colonies of streptococci have almost the same appearance as after 24

hours' incubation and usually remain inconspicuous. When examined carefully, however, a minute black spot may be made out in the centre of the colony, and this tends to become more marked after prolonged incubation. The microscopical appearance of *C. diphtheriae* grown on this medium differs only from those grown on Loeffler's medium in that the metachromatic granules are not quite so abundant; they are, however, sufficiently numerous to make the use of the diagnostic stains bases upon their presence perfectly satisfactory. Virulence of emulsions of *C. diphtheriae* was, in the case of 19 stains investigated, similar in every way to that of emulsions made from cultures grown on Loeffler's medium. (Andrewes and others, 1923)

The following advantages are claimed for this medium:

1. It is perfectly clear.
2. The amount of serum required is very small.
3. The colonies of *C. diphtheriae* and allied organisms having a distinctive appearance, negative records can often be seen without microscopical examination.
4. The isolation of *C. diphtheriae* is rendered much easier and more certain.
5. A larger number of positive results are obtained than when Loeffler's medium is employed.
6. The medium is not liquified by proteolytic organisms.

The Collection of Material for Examination.

The best material for laboratory examination is a portion of membrane removed from the affected region with sterile forceps: alternatively, a platinum loop can be used to remove faucial secretion, and this method has the advantage that the end of the loop can be bent at a right angle so as to reach the tonsillar crypts or other recesses. These procedures, however, imply that the bacteriologist has access to the patient and can make his cultures on the spot. In practice, it commonly happens that persons unskilled in bacteriology have to secure specimens and for this purpose a cottonwool swab on a stout wire is used. A stiff wire with a pledget of cottonwool firmly wrapped round one end for about $\frac{3}{4}$ in. The wire is kept in a narrow hard glass test-tube, and for convenience the other end is fastened to the cork which stoppers the tube. The tube with the contained wire and swab is sterilised by dry heat. In the first place it is essential that no antiseptics (e.g. in form of gargles, etc.) must have been applied within twelve hours, and the swab must be seen to come into actual contact with the faucial exudate and should be used with such gentle force as to remove some of it.

In swabbing contacts there is naturally no exudate to remove: all that can be done is to rub the swab thoroughly over the fauces; and this is true also of laryngeal diphtheria.

In obtaining material from the nose, the swab should be so introduced as to reach the turbinal bones, as these are the usual seat of membrane: it is difficult to control the procedure by actual inspection. Material from the eye and ear and from wounds is easy to secure.

There are two ways in which material may be examined for the purpose of presumptive diagnosis within 24 hours. The first is direct microscopic examination of stained films of the secretion, the second is cultivation on a suitable medium. Of these methods, the second is by far the more certain and must invariably be carried out, but there are reasons why, as a preliminary measure, the first should not be neglected. These reasons are:

(1). It sometimes happens that the diphtheria bacillus is present in such large numbers and in such typical form that a diagnosis can be made with almost as great certainty as from a culture. If the case is one in which the earliest possible provisional report is desired, there is a manifest saving of time when such a report can be offered from the direct film.

(2). In rare cases of true diphtheria, cultures may be negative although the bacillus can be seen in

the direct film.

(3). If direct staining be omitted, cases of Vincent's angina - a condition which may simulate diphtheria (as previously indicated under "Differential Diagnosis") - will be overlooked, since the ordinary cultural method does not reveal the characteristic organism of that affection.

The direct film examination - should only be used in cases of acute faucial diphtheria; in nasal and other cases the fallacies are so great that it should never be attempted, while in the case of carriers it is futile. Even in throat cases Neisser (1913) urged that a provisionally positive report should never be given unless the characteristic bacillus is abundantly present and unmistakable in its morphology. A negative result has no value, for in the majority of cases which prove culturally positive the direct film is negative.

As regards choice of culture medium, not one has established itself as superior to Loeffler's serum, or indeed to plain serum.

Methods of Inoculation - There are two ways of examining a culture; one is by picking off isolated colonies and the other by making a sweep of the mixed growth. These two methods will be discussed later, but the one which it is proposed to follow will determine how much material should be inoculated: if a mixed sweep is desired, the richer the inoculation,

the better; whereas if well isolated colonies are wished, the material must be thinly spread. Rich inoculation offers the better prospect of detecting the bacillus should it be sparsely present; thin inoculation affords the better chance of isolating it. As it is not known in advance whether the number of colonies will be large or small, it is well to inoculate one tube as thickly as possible, and a second more thinly from the first.

The swab, if dry, should first be moistened in sterile saline, or in the condensation water in the tube, and then rubbed thoroughly over the surface of the medium, care being taken not to break up the surface of the latter. The usual temperature for incubation is $37^{\circ}\text{C}.$, but Neisser (1897) states that, in order to get the full value of the method of staining associated with his name, the optimum temperature lies between $34^{\circ}\text{C}.$ and $36^{\circ}\text{C}.$ and should not exceed the latter limit or the reaction may fail. A positive culture examined after an incubation of between 5 and 6 hours at $37^{\circ}\text{C}.$ will probably show the bacillus, even though no colonies are apparent to the naked eye, and it will show little else. But at this stage of growth, although the bacilli are large and well formed, with barred or segmented protoplasm,

the metachromatic granules have not yet developed. These latter are stated to commence their development after about 6 hours of incubation, and at the end of 8 hours they are well formed and conspicuous, and it is a common experience that at this time a provisional diagnosis can be made. Neisser advises examination between the 9th and 24th hours for obtaining the greatest differential value for his stain. The question arises as to whether cultures which prove negative in 24 hours should be further incubated and re-examined on the second day. Although in many laboratories this is not the practice, there is evidence to show that, especially in convalescent cases of diphtheria, not a few cultures are found positive on the second day, though apparently negative on the first. Neisser (1913) gives figures as follows at the Frankfort Institute. Of 159 plates from recent diphtheria, negative on the first day, only one was positive on the second; whereas of 576 similar plates from convalescents no less than 55, or 9.5 per cent., showed diphtheria bacilli first on the second day.

The Examination of Cultures.

There are two alternative methods - in the first, suspicious colonies are picked off and examined individually; in the second, a film is made from a sweep of the mixed colonies on the surface of the culture. Isolated colonies present the advantage that pure subcultures can be more readily obtained, but it is often difficult to pick off a genuine colony at once, and moreover the earlier the cultures are examined, the more liable are the colonies to be confused with those of staphylococci or other organisms.

It commonly happens that the colonies are so closely set that it is not possible to pick them off singly. Under such circumstances the method of sweeping off a mixture of colonies is imposed upon the worker, and Neisser (1913) claims that it is actually more successful than the examination of single colonies, in the sense that a larger proportion of positive results is obtained, while the saving of time may be considerable. He supports this opinion by experiments carried out at the Frankfort Institute. Counted broth cultures of *C. diphtheriae* and *Staphylococcus aureus* were so mixed as to contain one diphtheria bacillus to 2,000 cocci. Serum plates of the

mixture were repeatedly examined by taking sweeps of the mixed colonies, and Neisser states that the diphtheria bacillus was never once missed - a result which he regards as impossible of attainment by any isolation method. This method has the advantage that large amounts of inoculum can be employed, and within reasonable limits, the thicker the culture is sown the greater the prospect of finding the bacillus. It may be added that, in examining a thickly sown culture on serum, it is often advantageous to take material from the edge of the massed growth, since here the diphtheria colonies have had the best chance of developing.

Wet staining - is carried out by gently distributing a trace of the culture in a drop of dilute stain on a slide and examining forthwith under a cover-glass, or, if preferred, as a hanging drop. A good dry high power, such as the Zeiss F objective, is perhaps to be preferred, but a 1/12" oil immersion is more commonly used. The stains which have chiefly been employed for wet examination are Bie's, and Pugh's acetic acid toluidine blue and also Czaplewski's stain; all these give good results.

The staining of dried films - Loeffler's methylene blue is very good. Neisser's stain is better for

rendering the metachromatic granules more sharply visible, and his second method, in which crystal violet is employed in addition to methylene blue, is an improvement on the original formula. Personally, I prefer Pugh's acetic acid toluidine blue which is a very useful and rapid single stain.

The characters of the diphtheria bacillus - these have been mentioned under Morphology.

There is usually little difficulty in determining the presence or absence of bacilli conforming to the classical picture of *C. diphtheriae*; doubts arise only when the bacilli depart in some respects from this picture. The polymorphism of the bacillus has been noted, and it is true that when the individual bacilli in a film are compared with one another they are found to vary greatly in form and size. Notwithstanding this, the general impression produced upon the eye by a diphtheria film is a fairly uniform one.

By far the commonest form encountered is the long and slender type, evidently thicker at one end than at the other, but in the young culture without pronounced clubbing. The mere shape of the organism suffices to distinguish it from bacilli belonging to other genera: singularly enough one of

the bacteria with which it is most liable to be confounded is the streptococcus. Certain streptococci, common in the throat, often assume, when grown on serum, a bacillary form of an imperfect kind with somewhat irregular staining, and Gordon (1903) has published microphotographs showing the striking way in which such cultures may mimic the diphtheria bacillus, though Neisser's stain at once dispels the illusion. The short type of *C. diphtheriae*, less commonly met with, presents greater difficulty in diagnosis. Though not so stout as *C. hofmannü*, it may be confused with that organism where a simple stain is alone employed. The chief difficulties, in any case, arise in distinguishing between *C. diphtheriae* and other corynebacteria. Such difficulties occur even in throat cultures; in cultures from the nose and elsewhere they are formidable. The metachromatic granules, in a well-stained film, are of the greatest help in distinguishing between *C. diphtheriae* and *C. hofmannü*. In a serum culture between 8 and 24 hours old, the former shows them in marked form, the latter practically never, or so feebly developed and in so few individuals, that mistake is scarcely possible, though even apart from the granules, the morphology of the two organisms is distinct.

tive to the experienced eye. These are the two species of *Corynebacterium* which chiefly occur in the throat, and this is the reason why Neisser's stain is of such great value in the morphological diagnosis of diphtheria. When the question arises of distinguishing between the diphtheria bacillus and corynebacteria other than Hoffmann's bacillus, this stain loses a great part of its differential value, for many other diphtheroids of no pathological importance may show metachromatic granules as well developed as in *C. diphtheriae*, or even better, though they do not appear so early. Thus we see that a presumptive diagnosis is likely to be correct in a case of sore throat or laryngitis, while in cultures from the nose, eye, or ear the margin of error is so much greater that the cautious worker will never pronounce a positive opinion. Even in throat cultures there exists the possibility that bacilli conforming in every morphological respect with the diphtheria bacillus may prove on testing to be non-virulent. In acute sore-throat this is seldom the case, though carriers of the non-virulent bacillus may suffer from simple tonsillitis.

But, in the case of contacts and carriers, the bacilli found prove so frequently to be non-viru-

lent (and, as it seems, harmless), that virulence tests should be carried out before any serious administrative steps are taken. It must be remembered that diphtheroids other than *C. diphtheriae* and *C. hofmanni* do at times occur in the inflamed throat, and that even the most experienced worker may at times return an erroneous positive diagnosis on morphological appearances alone, while it is well recognised that a negative report by no means excludes diphtheria. Further, in the convalescent case and the suspected carrier, errors are more frequent, as the non-virulent form of the diphtheria bacillus is more commonly met with.

The Immediate Virulence Test.

This is a method of testing the virulence of a culture without isolating the bacillus, and is based on the assumption that virulent bacilli, if present in a culture, will produce their characteristic pathogenic effect in spite of the presence of contaminating microbes. Glücksmann (1897) advocated the subcutaneous injection of an emulsion of the primary mixed culture into a guinea-pig. Havens and Powell (1922) have introduced an intracutaneous test - they picked and tested 210 colonies of *C. diphtheriae* from 17 throat cultures, all of which con-

tained virulent bacilli; 5 colonies, derived from 4 different cultures, proved non-virulent, indicating a chance of error of 1 in 42.

In the crude test a control animal receiving antitoxin is in all cases essential. In the ordinary subcutaneous method the death of the animal may be due to pyogenic cocci or other organisms present in the mixed culture. In the intracutaneous method, confusing results may similarly arise unless checked by a control. The method of Havens and Powell was checked by them on 509 whole cultures, 341 of which were positive and 168 negative on microscopic examination. Not one of the latter gave a positive cutaneous test: 305 of the cultures microscopically positive yielded evidence of virulence when tested cutaneously. In 295 cultures the bacillus was also isolated, and the cutaneous test compared as between pure and mixed cultures: the results were identical in 93 per cent. Their technique is shortly as follows:

After microscopic examination, the cultures (on Loeffler's serum) are suspended in saline, the dilution varying roughly with the proportion of bacilli found in the culture. The aim is to inject some 20 million bacilli in 0.1 c.cm. Two large

guinea-pigs, one of which has received 250 units of antitoxin on the previous day, suffice for testing 6 cultures. The tests are finally read after 72 hours: the criterion of virulence is superficial necrosis of the skin, absent in the control: mere discoloration or oedema count for nothing.

Force and Beattie (1922) have used an intracutaneous method similar in principle to that of Havens and Powell. They tested 247 crude cultures and in 202 of these they carried out both intracutaneous and subcutaneous tests. All tests were controlled by antitoxin-protected animals. They found that the intracutaneous was less often influenced by contaminating organisms than the subcutaneous method. No discrepancy was noted between the virulence of pure cultures isolated from the 'field cultures', as they call them, and that of the field cultures themselves.

Bull and McKee (1923) reported on the results they obtained, by Havens and Powell's intracutaneous method, during a search for carriers amongst some 4000 school children in Baltimore. They tested 198 crude cultures, and obtained evidence of virulence in 31 of these. From 26 of these 31 cultures, virulent bacilli were isolated in pure cul-

ture - i.e. the method proved correct in at least 83.8 per cent. of cases.

From 63 cultures which gave no evidence of virulence intracutaneously they failed to isolate virulent bacilli in every single instance. They consider that, had they relied on morphological evidence alone, there would have been such a high degree of error that little significance can attach to this form of diagnosis where carriers are concerned.

The Complete Diagnosis of the Diphtheria Bacillus.

It has been noted in the preceding section that the recognition of *C. diphtheriae* by morphological characters alone is no more than a presumption, likely, indeed, to be correct in cases of acute sore-throat, but under other circumstances beset with fallacies. The bacteriologist who has to report on a given swab should be supplied by the clinician with notes on a printed form describing the general nature of the case, the character of any exudate and the degree of suspicion attaching to it, or, if the case is a contact or a suspected carrier this fact should be noted. It is of great importance to state whether the swab is from the throat, or from the nose, eye, ear, or elsewhere. Even in suspected throat cases the presence of morphologically typical *C. diphtheri-*

ae cannot always be accepted as sufficient evidence of the nature of the case, and verification is desirable. The rule must be laid down that verification is imperative when dealing with swabs from the nose, ear, or eye, or in contacts or suspected carriers. The process of verification consists in isolating the suspected bacillus in pure culture, and then submitting it to certain routine tests, amongst which that of virulence is the most important and decisive.

Isolation of the Diphtheria Bacillus.

Whereas for the detection of *C. diphtheriae* a primary desideratum is a medium favourable for its growth, and less favourable for associated bacteria, almost the reverse is true when it is a question of isolating the bacillus in pure culture. It is a common experience that an apparently isolated and pure colony of *C. diphtheriae* on serum yields, when subcultured on agar, a mixed growth of streptococci and diphtheria bacilli. Serum is thus an unfavourable medium for isolation, agar is a good one, better still is Douglas' tellurium-trypsin-agar. No culture of *C. diphtheriae* should be deemed pure till it has passed the test of growth on an agar slope, for this at once reveals any admixture of pyogenic cocci. When, as is commonly the case, the colonies on the

primary serum culture are too crowded to allow of single colonies being picked off with certainty, it is necessary to remove a trace of growth from a spot where the bacillus is known to be present, dilute it, and plate it out. This may be done on agar, either by ordinary plate culture, or by a series of parallel streaks on two or more agar slopes. But it is here that a tellurium medium shows its especial usefulness and the medium devised by Douglas is particularly to be recommended owing to its transparency.

Tests to be applied after Isolation.

Having been obtained in pure culture, the organism is now to be submitted to certain routine tests in order to determine its true nature. These may be considered under the following heads:

Staining tests - These are necessary to ensure that a morphologically correct organism has been selected. A simple stain such as Loeffler's methylene blue is as good as any. Or Gram's stain, with prolonged washing in alcohol, may be used. Granule stains, such as Neisser's, are less suitable for cultures on agar than for serum cultures, so that if it is desired to employ these it is best to subcultivate again on serum.

Fermentation tests - Only three sugars need be em-

ployed in routine testing, namely glucose, galactose, and saccharose. A 1 per cent. solution of these sugars in peptone water or Hiss' serum water, with an indicator such as litmus, or Andrade's more delicate bleached acid fuchsin, will be found to give clear results, often apparent in 24 hours, though 4 days should be allowed to elapse before the final reading.

~~The test.~~ There should be evidence of good growth in the tubes pronounced to be negative.

~~Method.~~ The diphtheria bacillus is quite constant in its reactions: it produces acid in glucose and galactose, never in saccharose. Hofmann's bacillus is equally constant in producing no acid in any of the three sugars. The use of saccharose enables us to diminish the saccharose fermenters. If saccharose is not fermented, but glucose and galactose yield acid, the bacillus may be *C. diphtheriae* in its virulent or nonovirulent form: sugar tests do not allow us to distinguish between them. If glucose alone of the three sugars is fermented, the possibility of *C. diphtheriae* may be dismissed.

The Virulence Test - This is the only certain and satisfactory confirmatory test for the diphtheria bacillus, for no other species of the genus *Corynebacterium* occurring in man produces a fatal effect

upon the guinea-pig. Moreover, it possesses the advantage of discriminating between the pathogenic organism and its non-virulent ally, which no other test will do. Animal experiment is thus a necessary part of the complete diagnosis of *C. diphtheriae*, and is of fundamental importance in the recognition of carriers. There are two methods of carrying out the test, and they are equally good, though the second has the advantage of economy. In the first method, a living culture is injected subcutaneously into a guinea-pig, and it is an advantage to inject at the same time a control animal with the same dose, but with the addition of antitoxin, though this control is less essential than when an impure culture is injected. The growth from a whole serum slope should be emulsified in saline and injected, for the dose must not be too small, or minor degrees of virulence may be overlooked. If the organism tested is a virulent *C. diphtheriae*, the unprotected animal should die in from 1 to 4 days, with the following lesions:- Marked oedema at the inoculation site, enlargement of lymphatic glands, especially the inguinal and axillary glands, distension and congestion of abdominal vessels, and an exudate, which may be clear or cloudy and haemorrhagic, in the pleural cavities

and to a less extent in the peritoneum and pericardium, enlargement and congestion of the adrenals, this latter is the most constant lesion of experimental diphtheria. The proper test of virulence is death, and it is best to disregard mere illness or local swelling. A non-virulent bacillus will produce no effect even in the unprotected guinea-pig. The expense of sacrificing at least one animal for each strain investigated is largely overcome by the second method of testing, in which a much smaller dose of the bacillus is injected intradermally in the manner first introduced by Römer (1909). He tested three samples of diphtheria toxin and found that definite lesions were produced when the material was injected strictly intracutaneously in a dose of 0.1 c.cm. The amount which produced a local lesion was $1/250$ to $1/500$ of that which was required to produce a fatal effect after subcutaneous inoculation. He also noted that several injections of different dilutions could be carried out on one and the same animal. The lesions varied according to the dose. With quite small doses a slight redness and swelling appeared and lasted 3 or 4 days and was succeeded by a local loss of hair. With a medium dose there was a small wheal ending in necrosis, while with large doses

(1/50 m.l.d.) there was definite necrosis of the skin after 2 or 3 days. Römer's method was later elaborated by Neisser and Gins (1913) and by Eagleton and Baxter (1921). This mode of testing is not more delicate than the subcutaneous method, with which it runs closely parallel; but it has the advantage that a number of strains may be tested simultaneously upon a single guinea-pig, while neither this animal nor the control need die. For this reason the intradermal test is quickly replacing the older method.

The fermentation and virulence tests are adequate as confirmatory procedures for the complete diagnosis of *C. diphtheriae*, and as a rule there is no necessity for going beyond them. It might be mentioned, however, that there is the anaerobic test which depends upon the fact that *C. diphtheriae* is a good facultative anaerobe, while those diphtheroids which are chiefly liable to be confounded with it are somewhat strongly anaerobic. The test is carried out by making a glucose-agar shake-culture. After 48 hours the colonies will have developed. In the case of *C. diphtheriae* they will be present throughout the whole depth of the medium and may be almost absent at the surface; in the case of most other diphtheroids the colonies will be crowded at the sur-

face, but little or no growth will have taken place in the depth. In the present state of our knowledge Serological Tests for the recognition of the diphtheria bacillus must be regarded as of little value. (Medical Research Council, 'Diphtheria', Andrews and others, 1923, page 255).

The Schick Test.

The function of the Schick Test is to disclose the fact of the susceptibility or otherwise of an individual to diphtheria toxin, and therefore his susceptibility to the disease. It is thus applicable for indicating which members of a community require to be immunized if any danger of attack be present. It is also used for diagnostic purposes to differentiate carriers of diphtheria bacilli from cases of diphtheria. The former will give negative reactions and will not require treatment, while the cases will react positively. The method has not yet come into general use for this purpose.

The test consists in the intradermic injection of a very small but measured dose of diphtheria toxin. When no antitoxin is present in the blood, this injection will lead to a local inflammatory reaction which is described as "positive". When the person is immune no reaction will take place.

Preparation and Conservation of the Test Toxin.

although The amount of toxin is $1/50$ m.l.d. (minimum lethal dose) for a guinea-pig of 250 grammes weight, because this amount is the largest which can be used without producing reactions in persons who have sufficient antitoxin in the blood to give relative immunity. If smaller quantities be used some cases will give negative reactions, although they have too little antitoxin to provide immunity. A certain accuracy in preparation is essential. It may be said that the standardization of the toxin by the m.l.d. is clearly unsatisfactory in a test which is to a large extent quantitative and depends upon the union of toxin with antitoxin (Cowie, 1916; Glenny and Allen, 1922), and this standard, at present universal, will no doubt give place to one based, as usual, on the $L+$ dose. The $L+$ dose is the minimum amount of toxin which, injected with 1 unit of antitoxin serum, kills a guinea-pig of 250 grammes in five days. According to Zingher (1916) the toxin is a 6 day culture in sugar-free broth. This is carbolyzed and filtered and allowed to stand for 18 months to stabilize. The m.l.d. is then estimated in the usual way. When required for use the stock toxin is so diluted that $1/50$ m.l.d. is contained in 0.2 c.cm. This

diluted toxin should not be kept for over 24 hours, although the undiluted stock will last for 12 months. Part of the diluted toxin is heated to 70°C . for 5 minutes. The heating destroys the specific toxin, but does not injure the other constituents of the toxin broth which often cause confusing skin reactions (pseudo reactions). The heated toxin is therefore injected as a control. Zingher (1921) has suggested that the control should be 50 per cent. stronger, but Copeman, O'Brien, Eagleton, and Glenny (1922) could see no advantage in this alteration.

The test can be carried out most accurately with materials supplied from trustworthy sources in the form of outfits which merely require mixing together to give the requisite dilutions in a reliable form. Certain firms, for instance, supply a capillary tube containing such a quantity of undiluted toxin as will make the proper strength when blown into a bottle of sterile salt solution also provided. In the outfit is also a bottle containing heated control toxin already diluted. The materials supplied are sufficient for some 50 tests and in the undiluted condition will remain in a satisfactory state for several weeks.

Schick test toxin is also supplied diluted,

ready for use, in 1 ccm. and 5 ccm. sets in hermetically-sealed phials. The diluted toxin is issued for use as soon as it is received, although tests indicate that when stored in an ice chest it will remain potent for two weeks.

Technique for carrying out the Schick Test.

In order to obtain a true reading, the test must be carried out with great accuracy, and some slight practice is required for this.

Any properly fitting syringe of small capacity (1 c.cm.) is suitable. This is armed with a fine sharp needle $\frac{1}{4}$ in. long, a No.1 dental needle, or, better, a No.214 rustless steel needle (Wellcome) will be found satisfactory. One syringe is used for the toxin and another for the control. The injection of the toxin and of the control is made into corresponding areas of the flexor aspect of the forearms, the skin having been cleaned with a little spirit. The needle is introduced into the epidermis with the bevel up and is passed in parallel to the surface and so superficially that the bevel can be seen through the layers of the stratum corneum. The requisite quantity (0.2 c.cm.) is then injected with some little pressure, and a small white wheal will rise, its surface pitted where it is anchored down by

the hair follicles and sweat ducts. No dressing is required. The types of reaction which may be observed are four in number:

1. Negative.
2. Positive.
3. Pseudo (or Negative and pseudo).
4. Combined (or Positive and pseudo).

The alternative names for reactions 3 and 4 were suggested by Copeman, O'Brien, Eagleton, and Glenny (1922). These authors indicate the results by the following symbols: 1)-; 2)+; 3)X; 4)+X.

1. The negative reaction is recognised by an entire absence of reaction in both arms.
2. In the positive reaction nothing is observed on the control arm at any time, but on the test arm after 24 to 36 hours a red flush begins to develop and is at its maximum in 4 days. At this time it is a round circumscribed area measuring 1-2 cm. in diameter and slightly swollen. From its maximum it slowly fades in a further 7 to 10 days to a circumscribed brownish tint with desquamation of the epidermis. The pigmentation may remain for weeks.
3. The negative and pseudo reaction, as is to be expected from an allergic reaction, develops rapidly in 24 hours equally on both arms. It is a red flush

less circumscribed than the positive reaction. By the 4th day it has most disappeared, often, however, leaving behind a reddish or brownish pigmentation with a certain amount of desquamation.

4. The positive and pseudo reaction is a complex of both positive and pseudo reactions. The pseudo effect develops rapidly on both arms, and as this fades the true positive emerges on the test arm. In this way the test arm tends to acquire the characters of the true positive, while the control arm is clearing up. When both reactions are still present on about the 3rd day, that on the test arm is larger either obviously or slightly.

If it is only possible to take a reading upon one occasion this should be done not sooner than the 4th day, and not later than the 7th. In most cases the pseudo reaction will have died out in this time. The conclusions drawn from these results are as follows:

Negative and Negative and pseudo = Patient is immune.

Positive and Positive and pseudo = Patient is susceptible.

The chief danger of error lies in mistaking a positive and pseudo reaction for a negative and pseudo

reaction owing to a similarity in the size of the test and control. A susceptible person will then be looked upon as immune. Positive and pseudo reactors are, however, relatively rare.

It would be preferable, in many circumstances, to be able to read the result of the test in 24 hours instead of having to wait for 4 days. Cope-man, O'Brien, Eagleton, and Glenny (1922) made tentative readings in 24 hours in 400 cases and found that 5 per cent. proved later to be incorrect.

A method of judging susceptibility, founded on Romer's technique, has been introduced by Kellogg (1922, 1923). He injects $1/300$ L+ dose of toxin mixed with 0.1 c.cm. of patient's serum intracutaneously into guinea-pigs and, if a local necrosis results, it is presumed that the serum contains less than $1/30$ unit per c.cm. and that the patient is susceptible. The method is advocated as being free from the difficulties of pseudo reactions of the Schick test.

Zingher (1920) tested various Schick outfits in the U.S.A. and found that certain of these outfits, as supplied by commercial firms, showed variations in the diphtheria toxin content. He emphasised the importance of the following three points:-

- (a) a standard toxin with a dilution of the proper strength.
- (b) accurate technique in the injection by means of a good syringe and a suitable fine needle.
- (c) an accurate interpretation of the reaction.

He further stated that 2,200 Scarlet Fever patients in the Willard Parker Hospital had given negative Schick tests and were therefore not given any prophylactic injections of diphtheria antitoxin, and that not one of these patients developed diphtheria.

Michiels and Schick (1913) estimated that the amount of antitoxin which prevented the reaction after inoculation of their standard quantity of toxin ($1/50$ m.l.d.) was about $1/30$ unit per c.cm. These authors (1913) also tested 30 children by both Romer's and Schick's methods, and found that 15 who gave a negative Schick test had over $1/30$ unit, usually considerably over, while 15 who reacted positively had traces of antitoxin only. Kolmer and Moshage (1915) made similar tests with similar results. Twenty persons with negative Schick tests had $1/20$ unit or more, even up to 10 units, while 10 cases with positive reactions showed from $1/40$ unit per c.cm. to no antitoxin. Unfortunately, the large series of v. Gröer and Kassowitz, in which Schick tests and estimations of antitoxin were made in every case, was

marred by the use of a toxin for the Schick tests which gave a large number of paradoxical reactions. Nevertheless, if their figures (1919) are examined so far as they relate to young children, in whom paradoxical (pseudo-reaction) reactions are less common, it will be seen that there is a fair correspondence:

Table IX. Comparison between Römer's and Schick's methods for demonstrating the existence of immunity in man on the same series of cases.

Age	No. tested	Per cent. without antitoxin (Römer's method)	Per cent. positive to Schick's reaction.	Difference
0	143	16	4.9	+11.1
- 3mths.	57	29	28	+1.0
- 6	30	44	43	+1.0
- 9	32	60	59	+1.0
-12	68	68	70	-2.0
-2years	54	69	72	-3.0
-3	50	72	74	-2.0
-4	60	52	53	-1.0
-5	50	56	66	-10.0
-6	52	56	63	-7.0
-7	58	63	72	-9.0

It will be noted that, excluding new-born infants who are stated to react poorly to any cutaneous intoxication, the correspondence up to 4 years

is good, but in the next few years less close. The increase in the difference from the age of 4 years onwards (becoming more pronounced up to adult age) is due to the ever-increasing number of paradoxical reactions which, in the authors' series were observed first at the age of 12 months. From the information supplied, it is not possible to guess at the real significance of these paradoxical reactions, but it is clear that the number of positive Schick tests from the age of 4 years and upwards is rendered too high by reason of some of them being really pseudo-negative reactions. 80 per cent. were positive. At 9-17 months

Thus on the whole it may be accepted that, fundamentally, the results obtained by the Schick test are the same as those obtained by the direct measurement of antitoxin, but in practical application the former is at present more liable to error.

The percentages of positive results at different ages obtained by various authors may vary widely owing to differences in the population tested. The greater the density of the population examined, the greater number of Schick negative reactions Zingher (1921). Thus we find a high percentage of positive reactions in a rural population, and also in the better class population of towns. That at the end

Theobald Smith's classical work has shown that in guinea-pigs active immunity in a parent may be transmitted to offspring as natural immunity, and it is reasonable to suppose that the antitoxin present in infants has been acquired from the maternal blood. Further, v. Gröer and Kassowitz (1919) estimated the antitoxin incidence in infants from birth to twelve months, separating the bottle-fed from the breast-fed. At birth 84 per cent. had antitoxin, but up to three months the breast-fed remained the same (83.8 per cent.) while of the bottle-fed children only 57 per cent. were positive. At 9-17 months 44 per cent. of the former and 16 per cent. of the latter still showed antitoxic blood. They thus argued that the passive immunity diminished rapidly in bottle-fed children but was maintained in the breast-fed, and that this result was due to the passive transference of antitoxin by the milk, a conclusion which was entirely in keeping with Ehrlich's original experiments with abrin and ricin (Andrews and others, 1923). Bela Schick in Boston (1923) stated that the antitoxin of the newborn was derived from the mother. At the same lecture he pointed out that in adults and the newborn 85 per cent, were negative, i.e. immune, but that at the end

of the first year hardly 30 per cent. were negative, this diminished protection continues until the 6th and 7th year, at 16 years 85 per cent. were negative.

Park of New York (1919) has been the foremost on the American continent to advocate the use of the Schick test. As a result of a large number of tests, he gives the following statistics which confirm those obtained by Schick and correspond very closely with the age incidence of the disease:-

Under 3 months.....15% are susceptible

" 3-6 months.....30% " "

" 6-12 months.....60% " "

" 1-2 years.....70% " "

" 2-3 years.....60% " "

" 3-5 years.....40% " "

" 5-10 years.....30% " "

" 10-20 years.....20% " "

Over 20 years.....15% " "

	Gesamtzahl	davon	
		positiv	negativ
Neugeborene	291	16	275 = 93 Proz
1. Lebensjahr	42	18	24 = 57 "
2. - 5 Lebensjahr	150	95	55 = 37 "
5. - 15 "	264	131	133 = 50 "

Wir sehen dass beim Neugeborenen, der in 84 Proz.

Schutzkörper besitzt und in 93 Proz. negativ reagiert, eine prophylaktische serumeinspritzung gegen Diphtherie in den meisten Fällen überflüssig ist. Damit stimmt die namentlich in Findelhäusern gemachte klinische Beobachtung gut überein, dass Neugeborene bei vorhandener Infektionsmöglichkeit nur verhältnismässig selten an Diphtherie erkranken (Schlichter).

The above is quoted from Schick's original article on pages 2608, 2609, 2610, Muenchener Medizinische Wochenschrift (1913 July-December), and the figures correspond fairly closely with Park's table (1919).

It has been seen that, as a general rule, the passively acquired antitoxin rapidly disappears from the blood, so that at the age of one to two years most children are free. From this time onward, however, the antitoxin tends to return in an ever-increasing number of individuals. From analogy with other infective diseases it is difficult to suppose that antibodies can develop in the body except through the effect of the specific antigen, and Wassermann (1895) tentatively suggested that the production of these antibodies was an "acquired function" dependent upon infection with *C. diphtheriae*. He thought that persons might become immunized through

larval infections or as carriers, and such repeated infections would account for the increase of immunity with age. Andrews and others point out that "excluding the small number of actual cases of acute diphtheria, there are a number of 'larval' diseases due to the same bacillus which are at times very widespread. Further there are a number of persons who can hardly be said to be ill at all but who 'carry' the bacillus. Such carrying may be chronic or transient, and is found most frequently in communities affected by the acute disease. Thus, for instance, in a public school for children in New York, where diphtheria is more common than in London, transient or persistent infections, whether leading to marked symptoms or not, must be sufficiently common to be consistent with an infective origin for the antibodies described at different ages in such communities."

Glenny and Südmersen (1921) have shown that when an entirely non-immune animal is injected with a toxin-antitoxin mixture a very slight antitoxic response is developed, and that only after a long interval. This they term a "primary stimulus." But when such a slightly immune animal is again injected, a rapid and marked immunity develops. This is the

"secondary stimulus" The same result is obtained whether the initial slight immunity is "natural", due to obvious infection, or artificial inoculation. The differences in the responses to these two stimulus accounts for the well-known phenomenon that one individual reacts much more rapidly to inoculation than another. It depends upon the presence of a slight degree of initial immunity. Schick (1911) examined by Römer's method 24 children who had recovered from diphtheria. Eleven had recovered 12 months before and seven had a considerable amount of antitoxin, while among 13 who had recovered 2 to 3 years before, three only had slight antitoxin. From this result Schick concluded that antitoxin was formed after an infection but that it did not persist for a long time. According to Schick's figures, however, the period of persistence is not very much less than is found in many cases of typhoid or paratyphoid fever. Otto (1914) distinguished between convalescents who were carriers and those who were free from bacilli. Five non-carrier convalescents were found to have from 1/10 units of antitoxin per c.cm., while 8 chronic carrier convalescents had from 1/10 to more than one unit. The same point was made by Schürer (1919), Park and Zinger (1916) laid stress upon the effect of the

severity of the attack. Children with positive Schick reactions who passed through mild attacks without treatment remained positive the whole time and developed no antitoxin. On the other hand, long chronic infections led to antitoxin production. Ordinary cases, tested after the therapeutic dose of serum had been eliminated, showed positive reactions again in 65 per cent. of cases.

The effect of chronicity is also shown by patients with paralysis. Schick (1911) recorded 0.15 units per c.cm. in the blood of a child 5 weeks after recovery from diphtheria without treatment, and de Lavergne and Zoeller (1920) found antitoxin in 3 cases of paralysis, one of whom had not been treated at all. Thus it may be concluded that antitoxin is usually produced by an attack of diphtheria, though not to a great extent. The antitoxin does not develop immediately after recovery, and in certain circumstances tends to die out again after a number of months. It is most pronounced in convalescent carriers and chronic cases.

Dudley (1923) found that 84 per cent. of 50 cases of diphtheria had negative Schick reactions 3 to 6 months after recovery, and ascribed this immunization not immediately to the disease but to

slight infections undergone after returning to an infected school. the old boys, but this difference cannot be Zingher has published extensive observations (1921) which tend to show that antitoxic immunity as disclosed by the Schick test is due to unrecognised infection or is, as he calls it, a contact immunity. He tested 52,000 public school children in 44 public schools in New York and tabulated the incidence of negative reactions according to the degree of crowding in the areas tested. In a sparsely inhabited old-fashioned American neighbourhood he found 33 per cent. negative results, while in poor crowded districts inhabited by Irish and Italians no less than 84 to 80 per cent. were negative. Similarly in two private schools 21 and 25 per cent., and in a rural school only 15 per cent. were negative. Thus the greater the crowding, the greater is the number of immune children. in reaction due to bacillary an

Dudley (1922) investigated a school in which two outbreaks of diphtheria had occurred recently, to test the hypothesis which is now under consideration, namely whether the boys who had experienced both epidemics were more immune than the new boys. 831 boys were tested by Schick's method - of these 67 were in their first term and 15 had been

in the school for 4 years. The new boys were much less immune than the old boys, but this difference cannot be accounted for by the mere difference of age (11-12 years and 15-16 years). When the immunity of each term is examined it is seen that the increase is not continuous as it would be if it depended upon age only, but that it develops in two steps each of which is coincident with an outbreak with diphtheria. Thus the development of immunity did not take place in the absence of diphtheria, but only when diphtheria was prevalent, and the conclusion that it may be ascribed to unrecognised infection during the outbreaks seems to us to be justified. (Andrews and others, Med. R. Council, p.295-296).

The correspondence between immunity and pseudo (paradoxical) Schick reactions has been noted by v. Gröer and Kassowitz (1919). In their view, the pseudo is a true allergic reaction due to bacillary substance in the toxin used (foreign proteins in the toxin broth, Schick, 1913). Schick and Kassowitz (1913-14) looked upon the allergic reaction as due to a endotoxin and a hypersusceptibility of the tissues to it. Zingher (1921) found a much higher percentage of pseudo-reactions among immunes than among non-immunes, and argued that this implied a

development of an antibacterial immunity in addition to the antitoxic immunity. reached its maximum about

48 hours Dudley gives the following points in favour of the view that pseudo-reactions are associated with previous exposure to infection:- al condition was

"(1). The relative frequency of the reaction in immune as compared with non-immune boys. The course was,

"(2). The fact that 'new boys' only contained 9 per cent., as compared with the 24 per cent. of pseudo-reactors among the 'old boys', also suggests that this kind of reaction is correlated with a diphtherial environment. explained by supposing that a recent

"(3). A past history of diphtherial infection more than doubled the percentage of pseudo-reactions, whereas other infections, such as Scarlet Fever and sore-throat, had little effect in this direction.

"(4). A last observation which has a bearing on the relation of diphtheria to pseudo-reactions is this. Among the 176 negative and pseudo-reactors there were seven boys the severity of whose reactions on both test and control arms was at least three times as great as any others seen. Both forearms were equally affected in all seven boys. An area of about six inches in diameter became red, tender, and swollen with a brawny oedema. The local condition

"resembled a streptococcal cellulitis more than any-
"thing else. The reaction reached its maximum about
"48 hours after the test injections, remained station-
"ary for the next day, and then rapidly subsided dur-
"ing the next 48 hours. The local condition was
"accompanied by mild constitutional symptoms and the
"temperature rose to 99°F. or 100°F. The course was,
"therefore, exactly the same as the ordinary negative
"and pseudo-reaction except for its severity. The
"seven subjects of these giant pseudo-reactions had
"all recently recovered from diphtheria. The severe
"reactions may be explained by supposing that a recent
"attack of diphtheria had produced a marked degree of
"allergy towards some products of the bacillus that
"were present in the Schick test solutions. Sixty-
"seven per cent. of those boys, who had been combined
"reactors in contrast to 27 per cent, who were simple
"positive reactors, became immune within three months.
"Therefore non-immunes with a pseudo reaction general-
"ly become immune more quickly than those without one."

Schick (1913) observed that only those
nurses who had previously reacted positively to his
test developed diphtheria. Moody (1915) and Bundesen
(1915) saw no case of diphtheria in a person with a
negative reaction. Zuckermann (1915) placed children

with negative reactions in the same wards carriers throughout the winter and no case occurred among them, although diphtheria was prevalent in other sections of the hospital. L.T. Wright (1917) tested 12 nurses and found 11 with negative reactions remained well while one positive-reactor developed the disease.

In v. Gröer and Kassowitz's large series (1919) 6 children developed diphtheria and of these 5 had no antotoxin previously while one had 1/500 unit per c. cm. Blum (1920) recorded that from 1915 to 1920, in an institution, no child who had a negative reaction developed diphtheria. Renault and Lévy (1920) observed 39 carriers in a hospital in which diphtheria was prevalent: of these 25 had negative reactions and remained well, while 10 of the remaining 14 with positive reactions succumbed. Leete (1920) described 11 cases of diphtheria among 500 tested persons and all had positive reactions (1 doubtful). Armand-Debille and Marie (1920) investigated an orphanage infected with diphtheria and found that the 47 cases with negative Schick reactions did not develop the disease during the 4 months of observation. Park (1919) tested the reaction of 'thousands' of admissions to Scarlet Fever wards

during 4 years and no negative case ever got diphtheria, although 8 had a "moderate tonsillar infection" which in the case of 6 cleared up without treatment. It is not clear whether these cases were true diphtheria, larval diphtheria, or carriers.

Guthrie, Marshall and Moss (1921) experimentally inoculated 8 human volunteers with virulent diphtheria bacilli by swabbing their tonsils and pharyngeal walls with the virulent culture - 7 became carriers and of these 4 developed acute diphtheria. The 4 who developed the disease had positive Schick reactions prior to the experiment; the 3 who remained well had negative reactions. The person who failed to become a carrier was also a negative Schick reactor, and had received 250 units of antitoxin some time previous to the experiment.

The few instances of diphtheria occurring in children with negative Schick reactions are as follows: Shaw and Youland (1916) described the case of an 8 months' old baby. The Schick reaction was negative; no bacilli were found in the throat but virulent bacilli in the nose. Two days after the test the child developed diphtheria, and died of laryngeal involvement. Blauner (1921) gave an account of what purported to be typical diphtheria

(membrane on tonsils and pharynx, moderate temperature) in a dormitory of an orphanage. All inmates of the orphanage except 2 had negative Schick reactions either naturally or as a result of toxin-antitoxin treatment. In the dormitory were 29 children, and the epidemic started with 3 cases followed by 5 others at intervals of a few days. Seven of the cases had negative Schick reactions and one a positive. Virulent diphtheria bacilli were isolated from all. When the nature of the disease was recognised, serum was given and led to rapid recovery and, further, no other cases developed after the administration of prophylactic serum. Park (1921) was called in to see these cases and gives a different account of the facts, notably that the last 2 cases had no diphtheria bacilli in the throat. He examined the throats of 'some 50 healthy children' in the same and other dormitories, and 'fully 50 per cent.' were found to be carriers of virulent bacilli. He formed the opinion that these were not typical cases of diphtheria at all, but 'croupous tonsillitis' due primarily to streptococci, but superficially infected with diphtheria bacilli which were widely distributed throughout the whole orphanage (50 per cent. affected). He pointed out that the last 2 cases

had 'a follicular tonsillitis without diphtheria bacilli', while one of the cases 'had definitely started to recover before antitoxin was given.' Finally the bacilli isolated from the cases belonged to three seriological groups which is 'very strong evidence', 'proof' that they did not come from a single source. Thus there is considerable conflict of opinion as to the construction to be placed upon these observations. It would, however, appear that Blauner, when he wrote his paper, did not know of Park's investigations, and it is possible that in the light of these he may have altered his view. Dudley (1923) reports an interesting case of what would appear to be a case of clinical diphtheria in a Schick negative reactor. "There was a characteristic membrane which extended on to the uvula and soft palate, and the diphtheria bacilli isolated from his throat killed a guinea-pig on the second day with all the usual signs of diphtheria at the autopsy. "Sleeping in the next bed to this patient there has been a dangerous carrier. Owing to an error on the writer's part this carrier had been allowed to proceed on leave. Before he could be recalled he had infected his brother and sister with diphtheria, thus proving he was a carrier of the 'dangerous'

"type. Incidentally, primary swabs from this carrier's throat yielded an almost pure culture of virulent diphtheria bacilli. This attack of diphtheria in a boy with a negative Schick reaction can, of course, be explained by faulty technique or an error in diagnosis, but in the writer's opinion the case was one of true diphtheria arising in a boy with sufficient antitoxin to inhibit a Schick reaction, but not sufficient to withstand the massive doses of infection which were probably sprayed at him at intervals through the night from the dangerous carrier who slept in the next bed. If the exception to prove the rule in the Schick test exists, it is hard to imagine a better environment in which to find it than that just described. There were three more negative Schick reactors who, though at first diagnosed as diphtheria, were not considered subsequently to be so. The first one of these developed the typical erythema of Scarlet Fever the day after he was diagnosed as diphtheria. The medical officer of the fever hospital, to which this patient had been sent, reported that there were no grounds for believing that he was anything else than a carrier of virulent diphtheria bacilli who had contracted Scarlet Fever. In the second negative Schick re-

"actor the examination of his throat yielded virulent
"diphtheria bacilli, but 'fusiform bacilli and spiro-
"chaetes' were also numerous, therefore the most
"likely diagnosis was 'Vincent's angina' in a diph-
"theria carrier. The third had a suspicious looking
"membranous exudation confined to one tonsil only,
"but as the M.D. isolated from his throat were a-
"virulent it would scarcely be fair to include him
"among the definite cases of diphtheriaamong a
"population where negative reactors were six times
"as numerous as positive reactors, diphtheria selected
"13 of the latter to 1 of the former at a time when
"the chances each time were more than 6 to 1 against
"the random selection of a positive reactor. The
"probability of obtaining a special individual by
"chance from among 7, 13 times out of 14, is a negli-
"gible fraction, yet the Schick reaction survived
"this very severe test of its efficiency in distin-
"guishing the susceptible from the immune boys of
"the school." (Dudley, 1923).

Joe (Edinburgh Medical Journal, June 1927)
in a short survey of the Diphtheria Prevention work
in Edinburgh, points out that 5 cases had been noti-
fied as Diphtheria where the Schick reaction had been
read as negative, 2 of these cases were apparently

due to errors of technique or organisation and the 3 others were trifling infections. Further, only 2 cases "protected to the point of being Schick negative were reported as suffering from diphtheria. "Perhaps the smallness of the number in this class "has a certain significance, and the one case in 1921 "which the outside diagnosis was confirmed was a mild "one, occurred seventeen months after the final protective inoculation, and eight months after the "negative Schick test had been obtained." In none of these cases was a virulence test carried out, and prior to the giving of antitoxin no estimate had been made of the antitoxin content by securing a sample of blood or repeating a Schick test.

"It therefore appears to us to be established that in the great majority of cases the presence of antitoxin in the blood in sufficient quantity is not consistent with an attack of diphtheria, "and constitutes a protection against this disease." (Diphtheria Med. Research Council, 1923)

The actual amount of antitoxin required to protect against diphtheria does not vary greatly according to most authors. Karasawa and Schick (1910) held that more than $1/50$ unit per c.cm. protected; Michiels and Schick (1913) $1/30$ unit;

Behring (1914) $1/20$ to $1/100$ unit; Kolmer and Moshage (1915) $1/20$ unit. Kleinschmidt and Viereck (1913) thought that $1/20$ unit per c.cm. was sufficient to protect against ordinary risks but that this protection might be broken down by mixed infections or specially heavy exposure. Recently Bieber (1921) has arrived at similar figures as a result of experiments upon animals. He found that a guinea-pig with $1/300$ unit per c.cm. survived for 6 days after an inoculation which killed controls in 24 hours. Further, an animal with 5 units survived 100 fatal doses. He thus concluded that $1/20$ unit would protect against 1 fatal dose and thought that $1/100$ would suffice for a less severe infection.

(11) Glenny (1925) has pointed out that "while "0.01 c.cm. of an ordinary brew of strong diphtheria "toxin would seriously affect and even kill many "normal horses, others contain so much normal anti-"toxin that 10 c.cm. of the same toxin would not "harm them."

As ordinarily carried out the Schick test reveals the presence of about $1/40$ to $1/60$ unit of antitoxin per c.cm. and a negative case is assumed to have this amount at least and to be immune. This division of the population into immunes and non-

immunes is probably not so arbitrary as may appear. From the work of v. Gröer and Kassowitz (1919) it seems that the great majority of persons who do not give a negative reaction, i.e. who are susceptible, have less than $1/200$ unit per c.cm., the number between $1/50$ and $1/200$ being small. Thus to call a person with less than $1/50$ unit non-immune is for practical purposes justifiable because in most cases if they have not $1/50$ unit they have no appreciable antitoxin at all.

It is reasonable, in my opinion, to state that in order to establish a diagnosis of diphtheria in a Schick-negative person three conditions must be satisfied - namely:-

- (1). The clinical picture must be typical.
- (2). Virulent *C. diphtheriae* must be present in the throat or nose.
- (3). A sample of blood taken before a therapeutic dose of antitoxin has been given must show that there is sufficient natural antitoxin to ward off an ordinary attack of diphtheria.

Before describing my personal work in Surrey, I must place on record my high appreciation of the kindness of my chief, Dr. Joseph Cates, county medical officer of health for Surrey, for entrusting me with this work and for kindly permitting me to make use of my records of work done in the county.

A. Day Schools.

(1). A small outbreak of diphtheria commenced about the 25th February, 1927 among children attending Bandon Hill Council School, and it was decided to summon a meeting of parents at the school in order to describe the Schick Test and immunization against diphtheria. The meeting was held in the School Hall on Tuesday afternoon, the 15th March 1927 and was well attended, the Headmaster taking the chair. The work was described by the county medical officer of health and also by myself. It is interesting to note that one mother who had lately come from Edinburgh to live in Surrey, had some knowledge of Schick work and was anxious to help us in this anti-diphtheria campaign. On Thursday, the 17th March, all the children for whom consent forms had been signed were Schick tested, and the first reading was made on Friday afternoon, the 18th March (24 hours).

The second readings were taken on the 24th March - a seven days reading - and all children who were Schick-positive got an injection of 1 c.cm. of toxoid-antitoxin mixture, given intramuscularly in the upper arm into the Triceps muscle close to the bend of the elbow. Two further injections of 1 c.cm. of toxoid-antitoxin mixture were given at weekly in-

tervals to the Schick-positive children. The total number of children on the school registers was 336.

The Infants Department = 81; Mixed School = 255.

171 children were Schick Tested.

146 Schick positive.

25 Schick negative.

127 children received 3 c.cms. Toxoid-antitoxin mixture.

16 absent ill	} 9 had 2 c.cms. prophylactic;
3 refused to complete	

Altogether 20 cases of diphtheria were notified up to 31st March among the children at this school, and two of these cases were in children who had been Schick tested and before they were read, and of course they had no T.A.T. injections.

With the Easter vacation in April the disease appeared to have died out, and it was not until the 23rd May 1927 that another case was notified in the Infants' Department - a girl aet. 6 years - who had not been Schicked in March. A further case of the disease was notified on the 26th July, a girl aet. 8 years who had not been Schick tested.

It is interesting to note that the next case notified - on 28th July - was in a girl aet. 11

years who had been Schick-negative in March. I did not see this case owing to my absence on the Summer vacation, and Dr. Okell, of the Wellcome Physiological Research Laboratories, Beckenham made a bacteriological examination and reported as follows:-

N.B ----- 11/8/27

(swab received 6/8/27).

B. hofmanni isolated - Avirulent
Virulent K.L.B. absent in all cultures prepared from this swab.

In view of this report and the fact that the mother of this child told me that "there was nothing wrong with her daughter", I think that we are justified in regarding the case as definitely not true diphtheria. Since this case, only four further cases have been notified among the children of this school, and each of the four cases had never been Schick tested; unfortunately, one of these cases was fatal, a girl aet. 7 years who was a newcomer to the district (from Cambridgeshire).

On the 20th September 1927, Re-Test forms were sent out to the parents of the 127 children who had received 3 c.cms. toxoid-antitoxin mixture in March 1927 and the school was visited on Wednesday the 28th September to Re-Schick these children -

98 children re-Schicked { 2 of these children
 { Schick + suffered from
 { Diphtheria in March
 { 1927, and had no T.A.T.
 { mixture.

2 children Schick tested { one suffered from
 { Diphtheria in March
 { 1927.

9 refused re-Schick.

15 left school.

7 absent.

131 :- Total.

The first reading was taken at 48 hours on
 30/9/27 and the second reading at 9 days on the
 7/10/27 and the figures were:-

96 children re-Schicked = 62 Negative 34 Positive
 (Had 3 c.cms. T.A.T. (64.58%) (35.41%)
 March 1927)

2 children re-Schicked who had Diphtheria in
 March 1927 and were Schick positive and had no
 T.A.T. were = negative.

2 children Schick tested = 1 Negative; 1 Positive,
 a child who had diph-
 theria early in March
 1927 before Schick work
 was started.

34 Schick positive - 32 were given 3 additional
 c.cms. of Toxoid-antitoxin
 mixture making a total of
 6 c.cms. T.A.T. each.

- 2 left school having had 1
 additional c.cm. making a
 total of 4 c.cms. T.A.T. each.

The Schick-positive child who had diph-

theria in March 1927 had 3 X 1 c.cm. T.A.T. injections.

Immunity certificates were issued to the parents of the 62 children who were negative at the re-Schick test.

The headmaster of this school states 9/2/28, that he has had numerous applications for the Schick test from parents of new scholars and others at this school.

(2). Diphtheria broke out in Carshalton early in October 1927 and the headmaster of Stanley Road School requested that Schick work might be undertaken at this school. A meeting of parents was held on Monday afternoon, the 17th October 1927, in the large central hall of the school. The meeting was well attended and was addressed by the county medical officer of health and by myself, and it was carefully explained that the immunity conferred by T.A.T. injections might take several months to develop, but that the majority would probably become immune to diphtheria within three or four months. The usual consent forms were handed round to the parents who seemed to be quite enthusiastic about the work. The Schick test was carried out on Wednesday the 26th October 1927 and was, of course, only applied to

those children for whom consent forms had been signed by the parents or guardians. The first readings were taken on the 27/10/27 (24 hours reading) and the second reading on 3/11/27 (8 days reading) - one week's interval elapsed between the first and second injections of T.A.T. mixture, and two weeks' interval between the second and third injections of T.A.T. The number of children on the school register was 245.

201 children (inc. 6 staff) Schick tested.
 12 schick negative (including 2 staff).
 189 schick positive (including 4 staff).
 174 schick positive children received 3 X 1c.cm. T.A.T. (18 pre-school age).
 4 schick positive staff got 3 X 0.5 c.cm T.A.T. (reactions almost absent).
 8 absent (2 suffering diphtheria; 1 concussion)
 1 schick positive child - parents refused 3rd c.cm. T.A.T.
 1 " " " - parents refused 2nd c.cm. T.A.T.
 1 Left school (Portsmouth).

Of the 8 absentees, 5 had received 2 c.cms. T.A.T. and 3 had 1 cc. T.A.T.

The two cases of diphtheria were both of a mild nature. Altogether about 100 cases of diphtheria have been notified in Carshalton during the

months of October, November and December 1927, but the numbers of cases at this school fell off in December and only two cases of diphtheria have occurred among the Schick positive children who completed their ^{see} injections of 1 c.cm. each of T.A.T. (Toxoid-antitoxin mixture). The first of these two cases occurred in a girl aet. 8 years and was seen by me on 15/11/27 - she was a case of nasal diphtheria and was probably properly classed as a case of 'larval diphtheria'. This case was diagnosed exactly 5 days after the completion of the series of T.A.T. injections, and was apparently a mild case with little or no signs of toxæmia. The second of these two cases occurred exactly 4 weeks 5 days after the completion of the third immunizing dose of T.A.T. - this case was a boy, aet. 10 years, who was brought to a school clinic by his mother, who thought that the boy was suffering from influenza, and on examination he showed typical patching limited to, and covering, both tonsils, nose dry and clean, no rash, temperature was subnormal, tongue clean, no congestion of palate or faucial pillars, no glands palpable in the neck, swabbing of the tonsils left no bleeding surface behind but the exudate was very characteristic of diphtheria - the lad had a headache, slight discomfort on

swallowing, slight nausea but no vomiting. The private medical practitioner sent the boy off to the Infectious Diseases Hospital where he was regarded as a definite, but mild, case of clinical diphtheria.

It is interesting to note that this boy slept with his brother, aet. 8 years, in the same bed up to the date of his going to the Fever Hospital, and yet the younger lad remained free from diphtheria and gave negative swabs. These two brothers were both Schick tested on the 26/10/27, and while both were Schick positive, the older boy gave a much more brilliant + reaction than his younger brother. Further, the former boy was only kept in the hospital for $3\frac{1}{2}$ weeks and he made a rapid, uncomplicated recovery, but, unfortunately, no virulence test was made of the swab taken which was reported as positive K.L.B.

Another Schick positive child who had completed the immunizing course of 3 c.cms. T.A.T. in November 1927, suffered from slight sore-throat on 21/12/27 and the swab, taken by the family practitioner was reported as positive K.L.B, but the child was quite well within 24 hours and was allowed out of bed after 2 days, and further swabs were all negative. The case was not notified as it was not regarded as

clinical diphtheria and no antitoxin was given, the child was playing about in the garden within a week.

Medical Officer The local Medical Officer of Health reported that the outbreak was most probably spread from case to case and by carriers, and he was unable to trace any evidence of a milk infection. It is of interest to note that the majority of the notified cases occurred in a poor, overcrowded slum area where we found most of the refusals for the Schick work. Many of the notified cases were, however, in the pre-school age children, and a number among adult persons.

It is worth while noting that 33 cases of Scarlet Fever were notified during the months October-December 1927, inclusive at Carshalton.

" On March 7th 1928, the death occurred from diphtheria of a child aet. $5\frac{3}{4}$ years who had attended this school, and whose parents had refused the Schick Test in October 1927."

(3). Three cases of diphtheria were notified among children attending the Infants School at Cheam Common, early in October 1927, and it was decided to offer the Schick test and immunization for those children whose parents wished it.

A meeting of parents was held in the Parish

Hall close to the school on Friday afternoon, the 21/10/27, and the work was explained by the County Medical Officer of Health and by me, and the usual questions invited and answered, and once again it was emphasised that while some susceptible children might be rendered immune within 3 weeks of completing their third injection of T.A.T., others might not become immune under nine months, and that in any case Immunity Certificates could not be issued until a negative Schick test had been obtained. It was also stated that if any child developed diphtheria after completing the series of three prophylactic (T.A.T.) injections, that it was very likely to be of a mild nature. There were 129 children on the school registers.

(4). 98 children were Schick tested.
 90 " Schick positive.
 8 " Schick negative.
 88 Schick positive completed 3 c.cms. T.A.T.

This work was completed early in December 1927 and since then one of the children, aet. 8 years, who was Schick positive and completed the course of T.A.T. injections was notified on 21/1/28 as "Diphtheria." The swab result was positive, and the case was sent to the Fever Hospital by the private medical

practitioner - it was only kept at the hospital for a few days although there was the complication of Mumps, and the case was quite definitely not one of clinical diphtheria and Dr. Okell reported that the *C. diphtheriae* isolated from cultures prepared from the swab were avirulent to guinea-pigs. Further, the brother and sister of this case were Schick positive and had been given 3 c.cms. T.A.T.; they remained quite well. About the New Year, 1928, two sisters who had not been Schick tested, developed diphtheria and "were very acutely ill and nearly "died of the disease." Six other cases of diphtheria were notified in this district during December 1927, and January 1928, and all occurred in children who had not been Schick tested.

(4). A sharp outbreak of diphtheria occurred at Wallington, early in November 1927, among the children attending the Holy Trinity Council School, and it was decided to start Schick work there. A meeting was held on the 16/11/27 and addressed, as at the other meetings, by the County Medical Officer of Health and by myself - practically every parent present wished to sign consent forms.

The school was Schicked on the 18/11/27 and in view of the serious nature of the outbreak - there

had been six cases with two deaths - the readings were taken at 24 hours' interval and all those found positive were given 1 c.cm. of T.A.T. except for two asthmatic children who received the following injections:-

19/11/27 1/10th c.cm. T.A.T.

22/11/27 0.5 c.cm. "

25/11/27 0.5 c.cm. "

2/12/27 1.0 c.cm. "

7/2/28 1.0 c.cm. "

There were no reactions to any of these injections.

Two members of the teaching staff were Schick positive and one received 2 c.cms. and the other $1\frac{1}{2}$ c.cms.

T.A.T. All the persons Schick tested were re-read at 7 days' interval, and the margin of error at the 24 hours' reading was shown by the fact that 8 cases read as negative at 24 hours were positive at the 7 days' reading, circa 3 per cent. error (cf. Page 363, Medical Research Council, Diphtheria, 1923).

The total number of children on the registers in this school was 328:-

Boys	136	}	328
Girls	123		
Infants	69		

The record of the work is as follows:-

- Details of these 5 cases are as follows:-
- 316 Schick tested.
 - 307 Schick positive.
 - 9 Schick negative.
 - 265 Schick positive children completed 3 c.cms. T.A.T.
 - 2 Schick positive staff -
 - (1 had .5c.cm., .5c.cm., 1.0c.cm. T.A.T.
 - (1 " .5c.cm., 1.0c.cm. T.A.T.
 - (Reactions slight or absent. lived 1c.cm.
 - 6 Schick positive children developed diphtheria before completing.
 - 4 Schick positive children left school -
 - (2 had 2c.cms. T.A.T.
 - (1 " 1c.cm. " "
 - (1 " nil " "
 - 4 Refused to complete (2 had 2c.cms. T.A.T.
 - (2 " 1c.cm. " "
 - 23 Schick positive had 2c.cms. T.A.T. } -
 - 3 Schick positive " 1c.cm. T.A.T. } -
 - uncompleted owing to absence from school.

It is to be noted that 8 children were only tested on 25/11/27, and it is anticipated that most of these 26 uncompleted cases will be finished shortly. The school is divided up into three separate departments, Boys, Girls, and Infants, and of the 6 Schick positive children who were notified as cases of diphtheris, before completing the course of three immunizing injections of T.A.T., three were in the Boys' and

three were in the Infants' departments.

Details of these 6 cases are as follows:-

- (i). S.B. (Boys' Dept.) Schick + developed diphtheria on the first day of reading 19/11/27 and had no prophylactic (T.A.T.).
- (ii). N.H. (Infants' Dept.) Schick + similarly developed diphtheria on the 19/11/27 and had no prophylactic (T.A.T.).
- (iii). L.D. (Boys' Dept.) Schick + received lc.cm. T.A.T. on 19/11/27 and developed diphtheria on 24/11/27.
- (iv). K.S. (Infants' Dept.) Schick + received lc.cm. T.A.T. on 19/11/27; received lc.cm. T.A.T. on 25/11/27; notified as diphtheria on 2/12/27.
- (v). A.S. (Infants' Dept.) Schick + received lc.cm. T.A.T. on 19/11/27; received lc.cm. T.A.T. on 25/11/27; notified as diphtheria on 2/12/27.
- (vi). D.J.S. (Boys' Dept.) Schick negative at 24 hours' reading on 19/11/27; Schick + at 7 days' reading on 25/11/27; received lc.cm. T.A.T. on 25/11/27; notified as diphtheria on 2/12/27.

It is interesting to note that the three last cases are all brothers living in the same house, and that they were all notified as diphtheria at the same date. Altogether about 40 cases of diphtheria were

notified in the Wallington district during November and December 1927, but only one case was notified among children who had completed the course of three injections of toxoid-antitoxin mixture (T.A.T.). This latter case M.H. aet. 11 years (Girls' Dept.) was Schick positive and completed 3 c.cms. T.A.T. on 2/12/27; she was notified as diphtheria on 30/12/27 and was apparently a very mild case with slight exudate on one tonsil only, and "not ill at all" but she unfortunately developed Scarlet Fever, about the 14/1/28, while in the Fever Hospital. The mother of this child informed ^{me} that the medical superintendent of the hospital ascribed the mildness of the disease to the injections of T.A.T. It is interesting to note that her brother - case number (ii) - had diphtheria on 19/11/27, and is one of the six cases recorded above.

Two cases of diphtheria occurred in January 1928 among pre-school age children in two separate families where school children who had been Schick tested and immunized remained free from infection, although they had to be excluded from school as contacts until negative swabs had been obtained from the nose and throat. The only other cases of diphtheria among school children at this school were in those

children whose parents had refused the Schick test.

It is noteworthy that the great majority of the Schick positive at this school showed brilliantly intense positive reactions and that pseudo reactions were conspicuous by their almost entire absence - these facts are most probably not unconnected with the low incidence of diphtheria in this district during the past decade.

The social factor is also of importance as the area is almost entirely a high-class residential one

B. Institutions.

(1). A severe outbreak of diphtheria was reported in the early summer of 1926 at the Russel School, Ballands, Addington, with a total of 39 cases and three deaths from this disease; case mortality = 7.7 per cent. All the boys in the school, numbering 182 were Schick tested and of these 75 were Schick positive and were given 3 c.cms. T.A.T.

The ages of the boys range from 10 years to 16 years, and they come from good class homes in all parts of England. Since all the boys were rendered Schick negative it has been the practice to Schick test and immunize all the new-comers, and as there have been no refusals by the parents it has been

possible to keep this institution entirely free from diphtheria since July 1926. On 26/9/27 I was sent to this school to Schick test the new boys, 17 in number. The first readings were taken on the 28/9/27, i.e. 48 hours' reading and resulted in 16 Positive, and only 1 Negative, the latter having suffered severely from diphtheria two years previous to the Schick test. The 16 susceptible boys were given the usual three injections of T.A.T., 1 c.cm. being given to each lad at 10 day intervals.

This school was again visited on 1/2/28 for the purpose of re-schicking these 16 lads, and also to Schick test 15 new boys.

One of the former lads was absent suffering from Scarlet Fever and was not re-schicked, but it is expected that he will be tested again next term.

The results were:-

12 old boys re-schicked were now Negative (80%)

3 " " " " " still Positive.
(2 of these were read as "Negative" at the 48 hours' reading).

15 new boys were Schicked and of these -

12 new boys were Schick positive (1 read as "negative" at 48 hours' reading).

3 new boys were Schick negative (1 read as ? pseudo negative at 48 hours' reading).

A third reading was made of all these boys at 14 days'

interval, on 15/2/28, and this reading corresponded entirely with the second reading, 7 days' interval on 8/2/28. The 15 boys who were positive received three injections of T.A.T., thus 3 of the old boys have had 6 c.cms. T.A.T. The medical officer of this school, the teaching authorities, and the matron are enthusiastic about Schick work, and it is intended that all new-comers to the school will be Schicked and, if necessary, immunized, in the hope that diphtheria may be abolished from this institution for ever.

I may add that the healthy situation of the school, and the excellent arrangements for the health of the scholars, and the provision of wide spacing of beds in the dormitories, are worth recording.

(2). A severe type of diphtheria broke out in a reformatory school at Redhill early in May 1927. I was instructed to visit this Farm School in order to Schick the boys on the 23/5/27, and in addition 15 members of the Staff of the Institution expressed a desire to be Schick tested and consent forms were signed by them. The first readings were taken on the 25/5/27 (48 hours' interval) and again on the 31/5/27 (8 days' interval); owing to some urgency those read as positive at the 48 hours' reading were

given 1 c.cm. injection of prophylactic (T.A.T.) on the 25/5/27. Further injections of T.A.T. were given at weekly intervals between each injection.

The results were:-

	<u>Boys.</u>	<u>Staff.</u>
Schick tested.	96	15
Schick positive	46	8
Schick negative	50	7
Immunized (3c.cms.T.A.T.)	45	8

The following pseudo reactions were noted:-

Pseudo negative = 23 Boys; 2 Staff.

Pseudo positive = 2 Boys; 1 Staff.

One Schick positive boy left the Institution before completing the series of 3 c.cms. T.A.T., having only 1 c.cm. T.A.T. Of the 8 Schick positive members of the Staff, one had a very sore arm which, however, cleared up within 48 hours, and two others had moderately severe reactions to the second injection of 1 c.cm. T.A.T. It is interesting that the pseudo-positive member of the Staff showed the only really "bad arm" with swelling and congestion over an area extending to near the shoulder and partly down the forearm, but I must emphasise that the condition

cleared up entirely and left no ill effects behind. The boys' ages ranged from 14 years to 19 years and they came, mostly, from the poorest parts of London and other towns. Some of them were obviously of a low mentality, and all of them had, of course, been sentenced for various offences (47.9 per cent.+). The Staff consists of teachers and gardeners, cooks, etc., many of whom had apparently always lived in the countryside and thus accounting for their high rate of susceptibility to diphtheria (53.3 per cent.+). Further the teachers came from sheltered, better class homes and were therefore more likely to be susceptible. Altogether 30 cases of diphtheria occurred in this outbreak but no case was reported among any boys or Staff who had been Schicked, and there has been no case notified since this work was undertaken. No attempt has yet been made to re-schick the boys or Staff, and it will probably be impossible to carry this out satisfactorily owing to the boys finishing their sentences and getting their discharge from the Institution.

The percentages of Schick + and Schick negative are rather interesting and I repeat them:-

	<u>Boys.</u>	<u>Staff.</u>
Schick positive (per cent.)	47.9	53.3
Schick negative (per cent.)	52.0	46.6

The form of Immunity Certificate issued to all the persons found to be Schick Negative is as follows:-

NOTE: This form must be carefully preserved.

County Council

Public Health Department.

It is hereby certified that _____
of _____
has been:-

- x (i) Schick tested and found to give a negative response.
- x (ii) Immunized with toxoid-antitoxin mixture, and is believed to be immune to diphtheria.

If it is suspected at any time that he/she is suffering with diphtheria, will the medical attendant kindly send to:-

Dr. R.A. O'Brien,
The Wellcome Physiological
Research Laboratories,
Langley Court,
Beckenham, Kent.

- (i). Full details of the conditions found.
- (ii). Three c.cm. (or 1 fluid drachm) of the patient's blood.
- (iii). A swab from the nose and throat.

N.B. The swab should be taken before anti-toxin is given, but if there is any difficulty about obtaining the blood sample, the administration of antitoxin should on no account be delayed.

SECTION IV.

Signed

Treatment _____ for County Medical Officer
 Date _____

x. Strike out the alternative which is inapplicable.

The most important point is the isolation of the patient and the -----infection of everything used by him. Spoons, crockery, and the like must be set apart for special use, and frequently boiled. The isolation should be maintained until at least two consecutive swabs from the nose and throat have been cultures and reported negative. A long time is often required before this can be obtained, although in probably more than half the cases the cultures will be found negative within a week of the disappearance of the membrane. Only a comparatively small minority usually harbour the diphtheria bacillus after the fifth week is over, and the number of convalescent carriers detected after the ninth week is very small. It is large enough to make the management and treatment of carriers a most important question. It is certain, however, that persistence will be longer if other pathological conditions exist in the air passages such as obstruction, and diseased tonsils where the bacilli may lodge in the crypts and are not easily reached by whatever antiseptic is used. In a number of cases of persistent

SECTION IV.Treatment(a) Prophylactic:-

The most important point is the isolation of the patient and the careful disinfection of everything used by him. Spoons, crockery, and the like must be set apart for special use, and frequently boiled. The isolation should be maintained until at least two consecutive swabs from the nose and throat have been cultures and reported negative. A long time is often required before this can be obtained, although in probably more than half the cases the cultures will be found negative within a week of the disappearance of the membrane. Only a comparatively small minority usually harbour the diphtheria bacillus after the fifth week is over, and the number of convalescent carriers detected after the ninth week is very small. It is large enough to make the management and treatment of carriers a most important question. It is certain, however, that persistence will be longer if other pathological conditions exist in the air passages such as obstruction, and diseased tonsils where the bacilli may lodge in the crypts and are not easily reached by whatever antiseptic is used. In a number of cases of persistent

nasal infection foreign bodies of all sorts have been found. The old fashioned shoe-button, hay seeds, in fact anything that a child can push into its nostril and lose, may act sufficiently as an irritant to permit diphtheria bacilli to remain for a very long time.

Efforts to free an infected individual of persistent diphtheria bacilli have been numerous and various. Obviously one of the first should be to remedy pathological conditions or obstructions. Mechanical means such as massage of diseased tonsils has been advocated. Antiseptics are frequently used. Painting with a 5 or 10 per cent. solution of nitrate of silver well into the tonsillar crypts, or mercurochrome, have also been in favour. Heavy earths such as kaolin used as a dusting powder in the throat, the theory being that it draws as it were the bacilli out of the crypts by purely mechanical action. Some workers have recommended "pyocyanase" a ferment liberated into a broth culture by *Bacillus pyocyaneus*, used as a spray. "Over-riding" with other bacteria known to be antagonistic to diphtheria bacilli has offered great expectations. Living broth cultures of *Staphylococcus aureus* as a spray have been used for this purpose with some apparent

success. They are, however, not without danger. Lactic acid bacilli have been used in the same manner but the results are even less convincing. Lastly, but most important is tonsillectomy which is, sometimes, though by no means always, effective in obstinate cases. It is a wise precaution to immunize the patient first, or at least to test his immunity by the Schick reaction (Ker). Ker has also tried the steam spray with sulphate of zinc solution in troublesome cases, but without any evidence of success.

More recently a change of ideas regarding carriers has been apparent. It is only virulent diphtheria bacilli that are dangerous so that quarantine should depend upon the presence of virulent diphtheria bacilli. Estimation of virulence has long been a laboratory procedure. Until recently it has been a rather longdrawn out technique occupying at least 8 to 12 days, too long for routine use, especially in contact cases. The original virulence test was made by the subcutaneous injection of 48 hour broth cultures, pure strains from the field cultures having been first isolated. Roemer has shown the feasibility of an intracutaneous test in which the determination is obtainable within 48 to 72

hours. Wayson of the United States Public Health Service suggested the use of field cultures and this aspect has been fairly well worked out by Force and Beatty, Havens and Powell, and others who have shown that the presence of contaminating organisms in the original diagnostic culture will not interfere with typical results. This saves a good deal of time since pure cultures do not have to be isolated and in addition permits a determination in those cultures from which it is not possible to obtain pure strains.

This method is of advantage in assisting school authorities in the control of diphtheria, and is used particularly in America. Kelly and Potter in a survey of certain schools where diphtheria was prevalent have shown that as high as 47 per cent. of the carriers found were non-virulent and could therefore be released in three days, thus considerably shortening their period of quarantine, and decreasing the interruption with their school work. Obviously in clinical cases a virulence test is not indicated since the disease is test enough, but in contacts and in carriers it seems only reasonable to use the mechanism thus provided for reducing interference in their activities to a minimum.

W.T. Benson ('Lancet', 1923, p.897) gives

the results of his work in the treatment of the diphtheria carrier by means of detoxicated K.L.B. vaccines. He shows that this method had, "(a) no appreciable effect in hastening the disappearance of the diphtheria bacillus from the throat or the nose of the diphtheria convalescent; (b) does not prevent those cases of prolonged persistence to which after an arbitrary period of three months we apply the term 'chronic carriers'. Further, the administration of vaccines appeared to have no influence on the clinical course of the disease."

Commander Dudley (1923) has shown that diphtheria may spread in an institution when the carrier rate for virulent diphtheria bacilli is as low as 1 per cent. "In 11 carriers of virulent diphtheria bacilli the result of the Schick test previous to the discovery of the diphtheria bacilli was in every case negative. In 12 carriers of avirulent bacilli the Schick test was positive in four cases. The Schick positive carrier is unknown as far as the writer's experience goes. Over 300 throat swabs from 113 positive reactors have been examined at various times, but in only one case was a virulent diphtheria bacillus isolated. This carrier was a boy whose Schick reaction had

"been positive 10 weeks previously, but when he was re-
"tested a fortnight after it had been discovered that
"he was carrying virulent bacilli the reaction was
"found to have become negative. He is the case
"nearest to a Schick positive carrier which the writer
"has found".(Dudley). ~~taken, then, that a chronic~~
~~carrier of~~ "Carrying during the incubation period can-
"not be common because among the many Schick positive
"reactors examined several developed diphtheria who
"had recently been bacteriologically examined with
"negative results. One boy, who was a Schick posi-
"tive reactor, contracted diphtheria on the day fol-
"lowing an experimental throat swabbing which had
"yielded a virulent diphtheria bacillus, that is to
"say, this patient was found to be carrying diphtheria
"bacilli on the last day of the incubation period."
(Dudley, 1923). Further, Dudley (1926) showed that
the diphtheria carriers at Greenwich, Royal Naval
School, were of the dangerous type, as in two instan-
ces Greenwich School carriers are known to have in-
troduced diphtheria into their homes in other parts
of England. O'Brien and other experts suggest the
non-dangerous carrier is one in whom colonies of
C. diphtheriae are so scarce in the primary cultures
as to make isolation of pure cultures difficult or

impossible ('Lancet', 1923, p.195). "The isolation
 "of pure cultures from carriers was generally easy
 "at Greenwich, and often *C. diphtheriae* was the pre-
 "dominant organism in the primary throat cultures" (Dudley, 1926).

It may be taken, then, that a chronic carrier of virulent diphtheria bacilli invariably has a high titre of antitoxin in his blood, but it is not certain which of the two conditions precedes the other. Some authors hold that a person becomes a carrier rather than a case because he already has antitoxins in his blood, while others hold that he has antitoxins because he is an infected carrier. Infection must surely be the starting-point. This infection may be chronic, as in a case of rhinitis, or it may be transient and possibly repeated. Dudley thinks that immunity is developed as the result of the frequent reception and rapid destruction of diphtheria bacilli in numbers insufficient to cause actual clinical disease or to be detected bacteriologically as in the case of a carrier. He believes that in fact during an outbreak three "epidemics" are in progress simultaneously: (1) of clinical diphtheria; (2) of bacteriological carriers, and (3) an immunizing epidemic of subpathogenic natural

"inoculations" which are only recognisable by their results. He considers the relation of the reception of the pathogenic agent to that of its destruction is the determining factor in infection. If the reception goes on faster than the destruction, the virus accumulates till present in a sufficient dose to infect. This hypothesis agrees with the much greater danger of infection from proximity of susceptible individuals to the infective cases or the carriers during the night, since the eight or more hours of exposure allow a considerable accumulation of infective material, if the virus received is being destroyed at a relatively low rate. In the school concerned, the distance between the beds was only 1 foot 9 inches and it is claimed that Hoffmann's bacillus as well as the Scarlet Fever virus were spread mainly during the night. A plan showing the distribution of the beds in which cases of diphtheria occurred affords no evidence of spread in the dormitory, but other considerations and the probability of intermediate carriers lead the author to look upon the bedroom as the place where transmission usually occurred.

On the other hand strong evidence is brought forward that the first cases actually con-

tracted their infection in the classrooms by means of penholders sucked by dangerous carriers and then, during the next lessons, used by the members of another class in the same room. Virulent B. diphtheriae were experimentally deposited on a penholder in this way, and later, after lying for a fortnight in a cupboard, were cultured and shown to be both viable and virulent.

"The ultimate disposal of a persistent
"convalescent or healthy carrier is a problem of the
"greatest difficulty. My own practice is to detain
"the patient for at least 12 weeks in hospital,
"after which time the virulence of the bacilli is
"tested on small animals. Should they prove avir-
"ulent I allow the patient to go, advising the fre-
"quent use of antiseptic gargles. It is interesting
"that we have never had a return case traced to such
"a patient. If, on the other hand, the bacilli
"prove virulent, hospital detention should be main-
"tained for some months longer if necessary, and the
"question of tonsillectomy should be considered"
(Ker, 1921).

The London County Council arranged in June 1926 for the examination and treatment of chronic diphtheria carriers at Guy's Hospital by a throat

specialist and the hospital bacteriologist, with encouraging results.

The isolation of the patient having been effected, cultures should be taken from the throats of all contacts, and proved carriers isolated. The detected carriers may, of course, be either the cause or the result of the patient's infection; most frequently perhaps the latter has contracted his infection from one of them, as there is, no doubt, a very large number of persons who, immune themselves, harbour and distribute the diphtheria bacillus. We find in different years that a proportion varying from 10 to 15 per cent. of our Scarlet Fever patients are carriers of the diphtheria bacillus on admission to hospital.

Disinfection - The destruction of diphtheria bacilli upon contaminated objects, though naturally not to be omitted, has relatively little importance in preventing the spread of the disease. The bacilli do not exist to any serious extent outside the immediate vicinity of a patient, and therefore there can be little value in measures designed to disinfect dwellings in which diphtheria patients have been ill.

C. diphtheriae has little power of resistance to antiseptics. Loeffler (1891) stated that cultures

were killed in 20 seconds by 1:1000 corrosive sublimate, 3 per cent. carbolic dissolved in 30 per cent. alcohol, or 5 per cent. in water. With this limited power of resistance to antiseptics and the fact that a temperature of 58°C . is fatal, it is obvious that a possible infectivity of any clothes or utensils contaminated by a patient can be annulled with great ease. When the disease is spread by milk or by contaminated articles such as drinking vessels, the chief object in view should be not merely to destroy these bacilli, which is easy, but to find out and remove the source from which they became infected. As stated previously, Dudley (1923) has shown that spread may occur by the common use of pencils and pens, and if there is any such possibility, these objects should be sterilized and made personal to each child.

The carriers being dealt with, disinfection carried out, and the milk supply investigated, it only remains to decide what to do with the contacts whether they be carriers or not. The debated point is, should immunization be practised or not?

Immunization - There are two methods of immunization possible in the prophylaxis of diphtheria, the injection of antitoxic serum, or inoculation with toxin-

antitoxin mixture (or toxoid-antitoxin mixture). The first of these confers merely a passive immunity of short duration, whereas the latter will give an active immunity which is likely to last for some years. But, as time is required to secure results by the second of these methods, it is not suitable for dealing with actual contacts, and for the purpose at present under discussion we must employ prophylactic injections of serum.

The argument used against this system of protection is that it causes the sensitisation to horse serum of a large number of persons who at some later date may, if serum is given them for any purpose, develop acute anaphylactic symptoms. Fatal anaphylactic shock is a very rare phenomenon and the other manifestations are more distressing than dangerous, and Ker had no hesitation in recommending prophylactic injections, especially for small children and other close contacts, whether they were carriers or not. In the Fever Hospital wards, where the children are under an observation more constant than can be managed in a private house, Ker only gave protective injections if more than two cases appeared in quick succession in one ward, though detected carriers were always injected. The protection given

by antitoxic serum lasts for three weeks and becomes efficient after about 24 hours. A few patients therefore will develop symptoms of diphtheria within a day of the injection. In Ker's experience of over two thousand of these injections, only four patients showed signs of the disease shortly after inoculation, one within 12 and three others within 18 hours. Four other patients contracted diphtheria two on the 22nd day, one on the 25th, and one after 5 weeks. The dose given in every case was 500 units only, which is smaller than that usually employed in many American hospitals, in which prophylactic injection is practised as a routine on the admission of every patient. "This small dose has proved in my experience effective, and serum sequelae have been almost unknown, even a slight rash being regarded as a genuine curiosity" (Ker, 1921).

In hospitals in which prophylactic injection is employed as a routine for all children admitted, the preliminary application of the Schick test would be an advantage and would prevent many unnecessary injections.

Andrewes and others (Medical Research Council, Diphtheria, 1923, page 352) summarise as follows:- "The prophylactic injection of antitoxin

"should, therefore, be looked upon as a weapon for
 "use in serious emergencies, which are rare, and
 "even then as a palliative which may leave the state
 "of emergency to develop again in 3 weeks. It will
 "be a matter for individual consideration whether in
 "any particular case it is not more satisfactory to
 "proceed at once along the lines of separation and
 "active immunization of susceptibles by means of
 "Schick's test and toxin-antitoxin mixtures rather
 "than to establish a sense of security which is some-
 "times false."

As a mechanism for decreasing morbidity
 Schick and Park recommend "active immunization" in
 those individuals who lack natural antitoxin. Ac-
 tive immunity must be sharply differentiated from
 passive immunity. Passive immunity is conferred by
 injecting an anti-body containing serum derived from
 an animal that has been actively immunized. It is
 efficient, as stated above, after 24 hours, and the
 time of absorption depending on the route of injec-
 tion. Its duration is brief and, as previously re-
 corded, lasting not longer than two or three weeks.

Active immunization, on the other hand, is
 the reaction in the individual himself against some
 foreign poison. It takes longer to develop, from

three weeks to nine months; its duration is much longer, perhaps permanent; and the amount of antibody present is out of all proportion to the amount of antigen used. In a few instances attempts at "immunization by injecting vaccines of diphtheria bacilli or 'endotoxin' have been tried without any "apparent success." (Park and Zingher, 1916).

The use of toxin injections in man has been largely neglected owing to the belief that they are likely to produce paralysis, and also on account of the obvious danger of producing other severe toxic reactions. Dzierzgowsky (1910) experimented by injecting himself with numerous increasing doses of diluted toxin without ill effect. Magyar and Schick (1913) injected 30 persons who had positive Schick reactions with 3 small doses of unstandardised toxin, and did not observe that the reaction became negative.

The use of toxin-antitoxin mixtures for the preventive inoculation of man was first suggested by Theobald Smith (1907) as a result of his successful experiments upon guinea-pigs. He thought that under-neutralised toxin would be undesirable owing to a danger of paralysis, and then (1909) carried out experiments to show that exactly neutral mix-

tures were effective upon guinea-pigs although less effective than when free toxin was present. He again proposed that this method should be tested upon man. The first attempt to immunize considerable numbers of human beings was made by von Behring who examined various combinations of diphtheria bacilli, toxin and anti-toxin; the method settled down (1913) into the one that has been in general use until recently in Austria and Germany. Broadly speaking, this consisted in the use of an under-neutralised mixture of toxin and antitoxin - i.e. one in which there is slightly more toxin present than can be neutralised by the quantity of antitoxin. The mixture is therefore toxic to animals. Dr. van Boeckel (1924) in his report to the League of Nations writes:-

"We do not know the exact composition of von Behring's T.A. mixtures. The fact that they are administered intradermally is not calculated to simplify the technique, which is rendered still more complicated by the fact that the proper sensitising dose has to be discovered before the vaccinating dose can be administered."

This method seemed to be rather difficult to carry out in practice and is falling into disuse

in its original home where American mixtures and practice have recently begun to attract attention.

A new and brilliant chapter was opened by Park and his co-workers in New York in 1913. They first used a mixture of toxin and antitoxin containing in 1 c.cm. 3L + doses of toxin, under-neutralised to such an extent that doses of from 1 c.cm. to 5 c.cm. produced paralysis or death in guinea-pigs. Within the past few years, Park has replaced his original mixture by a second toxin antitoxin containing in 1 c.cm. only 0.1L + dose of toxin, though in each cubic centimetre of this mixture the same amount of toxin is left unneutralised as in the original 3L + mixture. This second mixture was tested on man in parallel with the original one and gave fewer local reactions, whereas its immunizing power was probably better. It is now in routine use in New York and most of the United States of America. The technique consists of giving 3 injections at weekly intervals of one c.cm. amounts of the prepared mixture subcutaneously at the insertion of the deltoid muscle.

The two American accidents in active immunization occurred apparently with preparations similar to Park's original mixture. It is now possible to

make some statement about the first of these accidents, that at Dallas, Texas, in 1919 when fifty severe reactions and six deaths occurred due to an excessive amount of toxin in the mixture.

A certain volume of toxin added to a certain volume of antitoxin may give a neutral mixture - i.e., one that is harmless to guinea-pigs when injected. But if the toxin be divided into two portions, the first of which is added and thoroughly mixed with the antitoxin, while the second portion is added subsequently, a toxic mixture may result. This is the well-known Danysz phenomenon.

The original tests of the mixture used at Dallas had been carried out thoroughly and were apparently completely satisfactory. Owing to an unfortunate mistake the applicability of the Danysz phenomenon to the mixture in question, when, after the first tests the proportions of toxin and antitoxin had to be adjusted, was overlooked, and a toxic mixture resulted. The second of these accidents (Concord 1924) at Bridgewater, Massachusetts, was caused by the accidental exposure of a mixture to intense cold for several days, when sufficient toxin was released to cause severe reactions in several dozen children. There were, fortunately, no deaths.

The explanation of this phenomenon has been given by Glenny and co-workers (1925) in this country. Now that this risk is recognised it is very unlikely that this same mishap will again occur. A very large number - probably several hundred thousand - injections of these American mixtures have been given with complete safety except in the two well-understood instances mentioned, and with excellent immunizing efficiency.

With regard to the Vienna (Baden) accident ('The Lancet', Oct. 3rd, 1925, p.713) it has apparently been impossible to obtain for examination in England a specimen of the mixtures used, so that no close comparison of the Vienna lethal preparation with the American or English mixtures has been possible. It is not easy to deduce from the published account by Helmreich (1925) exactly how the mixture was constituted, although it is clearly stated that it was made of toxin and antitoxin. Presumably the aim was to produce a mixture similar to those used by Park. If, as is suggested, a non-toxic mixture of toxin and antitoxin dissociated under ordinary conditions of storage and became toxic this phenomenon has not been met with in the very extensive experience of American and English workers. Busson

(The Vienna Antitoxin Controversy, 'Lancet', 1926, ii, p.1074) bases his statement on the supposition that all the phials contained atoxic mixture when issued, but that owing to the influence of heat or cold or other agent during storage, the mixture had dissociated so that toxin was released, and the mixture became highly toxic. There was evidence that the phials contained different quantities of toxin, owing to the varying course of the symptoms in different children. There were fallacies in the guinea-pig test made during the official investigation, and the tests applied for the presence of serum were fallacious. Grassberger's experiments show that an increase of toxicity can be obtained by the use of acids, alkalis, and by freezing. Toxoid + some small amount of antitoxin is preferred in this country - it appears to be equally efficient as an immunizing agent and has the great advantage of being safe, even if dissociation should occur as the result of exposure to extreme cold or to the action of any other physical or chemical agent. ('Lancet', 1926, ii, p.1074).

Glenny, Pope, Waddington and Wallace report (J, Path, and Bact., Vol.28, 1925, p.473) on the action of phenol on mixtures of toxin and antitoxin:-

"Certain strengths of phenol around 5 per cent.
"cause a greater relative destruction of dilutions
"of antitoxin than of toxin, and the addition of this
"amount of phenol to a non-toxic mixture may render
"it toxic. The local concentration of phenol that
"occurs when mixtures are frozen probably causes a
"similar effect. Toxin-antitoxin mixtures both
"3L + and 1/10thL + were frozen by us at two tempera-
"tures (1) at about -6°C . by means of salt and ice
"and (2) at -30°C . with carbon dioxide snow and
"ether. Two out of five of the mixtures at the
"higher temperature and all tested at the lower
"temperature precipitated after freezing and thawing.
"We did not find any increase in toxicity in any of
"these mixtures after freezing. It would appear
"that different effects were produced by freezing
"mixtures according to differences in the constitu-
"tion of the mixtures. The simplest explanation of
"increased toxicity occurring in any given mixture
"after freezing appeared to us to be that the local
"concentration that occurred on freezing and thawing
"a solution brought the toxin-antitoxin complex into
"contact with a high concentration of phenol or other
"substance which destroyed the antitoxin or rendered
"it insoluble. We know, however, that toxin is

"partially destroyed by high concentrations of phenol.
 "If our explanation is correct it should be possible
 "to demonstrate that concentration of phenol such as
 "would occur on freezing a mixture originally con-
 "taining 0.5 per cent. phenol must destroy antitoxin
 "to a greater extent than toxin. A toxin-antitoxin
 "mixture with 3L + doses of toxin so neutralised
 "with antitoxin that the mixture was almost non-
 "toxic to guinea-pigs, an intradermic reaction was
 "produced by 0.2 c.cm. of the undiluted mixture but
 "not by the mixture diluted 1 in 10. To this mix-
 "ture we added various amounts of phenol from 1-9 per
 "cent, phenol. The results showed that 1-4 per
 "cent. phenol were non-toxic, but that those with
 "5-7 per cent. phenol were very toxic."

Again, in 1927, two accidents with toxin-antitoxin mixtures were reported in Russia and China. The Russian accident was apparently due to the injection of toxin in mistake for toxoid, and was caused by laboratory error. The Chinese accident is in an entirely different category. The number of patients had outrun the supply of prophylactic, and a native orderly had thought to increase the bulk of the mixtures by adding sterile distilled water. The deaths and sequelae that ensued were the result of

of streptococcal infections, due almost certainly to a contamination of the diluent. The Therapeutic Substances Act (August 7th, 1925) lays down the standards governing the preparation of diphtheria-prophylactic, but it seems that the chief line of defence in the avoidance of accidents in this country lies in the laboratories where the material is prepared. In many, if not all, respects it is an advantage that practically the whole of the diphtheria prophylactic employed in this country is derived from one source, and is prepared in a laboratory that has a high reputation for efficiency, thoroughness, and scientific endeavour and that, moreover, for the last two or three years has only distributed toxoid-antitoxin mixtures.

Detoxicated Toxin

The next development in the history of immunization was the use (with antitoxin) of toxin which had been so treated as to deprive it of most or all of its original toxicity while leaving its immunizing power still high. In this "toxoid" class come the aged toxin, etc., used by Park (1923), the formalinised "toxoid" made by Glenny and Hopkins (1923) and used by O'Brien and co-workers in England, and the formalinised "toxoid" or "anatoxin" used by

Ramon and colleagues (1924) in Paris. about 0.1 c.c.

Theoretically, preparations of this toxoid class should be preferable to Park's first or second mixtures, for they can be made completely atoxic to the guinea-pig and they are free from the additional complication of having antitoxic serum present.

These toxoids are being tried cautiously on the three countries mentioned; it is possible that they will eventually entirely replace the other prophylactic preparations, but it is too early yet to predict this with any certainty. The immunizing power of toxoid is high, but it is apparently rather liable to cause reactions when injected. The concentrated toxoid preparations of Watson and Glenny (1924) may be added to this class; though they produce a high immunity in animals and are atoxic, they have not yet been used in human practice to any large extent.

Another class of prophylactic is represented by the toxoid-antitoxin mixtures made and used widely in England. Glenny and his co-workers (1924) discovered that if one makes a toxin non-toxic by treatment with formalin - a preparation which has high immunizing power - and adds a certain volume of antitoxin to the toxoid, the immunizing power of the toxoid is not materially reduced. A typical mixture

of the kind would contain in 1 c.cm. about 0.1 c.cm. of "toxoid" made by formalinising toxin with an original minimum lethal dose of between 0.002 c.cm. to 0.001 c.cm. until the lethal dose was reduced to about one-hundredth of its former value, or better, to the stage where 5 c.cm. produced no symptoms in guinea-pigs. In addition to the toxoid, the mixture contains a quantity of antitoxin varying from 25 to 50 per cent. of the amount corresponding to the original neutralising value of the toxin. The mixture which gives the best immunity index is the one chosen for use. This combination is non-toxic to guinea-pigs, gives excellent immunizing results in these animals, and is apparently a "safe" mixture.

Many thousands of doses have been given during the past three years. Even five or six years ago, when the first toxin-antitoxin mixtures of the American type were used in England, the standard of toxicity adopted was below that permitted by the official Washington regulations. These latter allow the use of mixtures of which 1 c.cm. will produce paralysis in all the animals injected and death in a small number.

It was found by the aid of the Glenny - Allen (1923) "immunity index" that it was possible

to prepare toxin-antitoxin mixtures of such low toxicity that 1 c.cm did not produce paralysis or death, while a dose of 5 c.cm, would cause paralysis or death in either none or at most a small percentage of the guinea-pigs injected, and yet would rapidly produce immunity. Most of the published work in England was apparently done either with mixtures of this type or of the toxoid-antitoxin type previously mentioned. These latter preparations appear to have a double margin of safety. They contain toxoid mixed with a quantity of antitoxin and thereafter diluted ten times. The toxoid itself is almost or completely devoid of toxicity. This change from toxin to toxoid is apparently an irreversible one, for in all the intense study of this field by various workers during the past few years there has arisen no evidence suggesting that toxoid in this condition can revert to toxin. Even if this very unlikely change took place and some of the toxoid were reconverted to toxin, the mixture contains sufficient antitoxin to neutralise a large amount of toxin.

Lines of Future Progress.

With regard to possible progress in the future, the main effort is being directed along three

lines. The first is to provide active immunization for children of pre-school age who are likely to be exposed to diphtheria. Most of the work hitherto has been carried out with children aged 6 and upwards, but a great deal of diphtheria occurs in younger children. Hutt emphasizes the importance of anti-diphtheria measures at Child Welfare Centres and gives records of his work in Holborn ('Lancet', 1925, ii, 962-965).

The second is to increase the immunizing efficiency of the prophylactic so that one could obtain the same efficiency of immunization, with two injections or even one, which now one obtains with three. Along these lines progress must be slow. It is easy to make immunizing mixtures of very high efficiency in the laboratory. Thus Glenny and co-workers have described a preparation which will make animals negative to the Schick test in 12 days, but the transfer of such work to the human practice must be slow, for the greatest caution is necessary. The other effort is to shorten the number of tests and injections by combining them. Park is directing effort to a mixture which when injected will indicate the response to the Schick test and at the same time will act as an immunizing agent.

A. Ceconi. In England there are at present (1926) being tried mixtures which will, when injected intracutaneously into animals, produce a Schick test reaction, and at the same time supply an efficient immunizing stimulus. One can thus, when each immunizing intradermic injection is given, get an indication of the condition of the patient and know exactly when to cease the injections. R.A. O'Brien ('Lancet' 1926, *ii*, p.616-617).

Characteristic energy has been shown by some public health departments in America and large numbers of children have been tested and the Schick-positive (susceptible) immunized with toxin-antitoxin mixtures (T.A.T.). Moreover, the subsequent histories of the children have been followed up as regards immunity and attacks of diphtheria.

In March, 1922, Park reported that among 90,000 children in New York who were known to be Schick-negative (Immune), either naturally (57,000) or as the result of immunization (33,000), 18 cases of diphtheria had occurred, as against 56 cases among 90,000 untreated control children; a rate or morbidity reduced to one-quarter. Dr. John Ceconi now records (Report of Progress on the Schick Toxin antitoxin Activities of Boston Health Dept. by John

A. Ceconi, M.D. And a Statistical Report on the same by Paul Eaton, M.D. Boston Med. and Surg. Jour., cl xxxviii. No.4, pp. 94-99, Jan 25th, 1923) the results of about 26,000 Schick tests made in Boston during six months in 1922. Of a series of 20,713 children tested 19,148 came up for examination and the results were "read"; 9983 were found to be Schick-positive (susceptible) and of these 7013 (70%) received three T.A.T. doses. The results of applying the test again ("re-Schicking") six months later were known in the case of 300 at the date of publication and showed a change from positive to negative in over 95 per cent. What change in immunity would have occurred naturally without inoculation can only be inferred from the general results of the Schick test, but there is no reason to suppose that it would have been considerable.

The prevalence of diphtheria bacilli at the period covered appears to have been very low. Examination of swabs from 1663 children who were Schick-positive yielded 6.2 per cent. of cultures of *B. diphtheriae*, all of which were non-virulent, and 1000 Schick-negative children yielded 5.7 per cent., also all non-virulent. The stimulus to the production of natural immunity by contact does not

therefore seem to have been present to any great extent. It is pointed out that the effect of immunization is not an increase in the number of carriers as some have feared would be the case. In a statistical report on the same date by Dr. Paul Eaton it is stated that the percentage of susceptibles may vary from 15.2 to 72 per cent. in crowded and thinly populated areas respectively. The cause of "pseudo" or "protein" reactions following the Schick test inoculation is not attributed mainly to a sensitiveness to the protein of the bacillus itself, but experiments on the point are stated to be in progress. The work in Boston is being carried out with a staff of eight physicians and seven nurses, and is presumably being continued with equal vigour. The effect of these attempts to prevent diphtheria by immunizing the Susceptible should be still more apparent in a few years. Zingher (1921) gives 3 doses of 1 c.cm. of toxin-antitoxin at intervals of 1 week, but in order to economise time in treating large numbers 2 doses of 1.5 c.cm. were also tried though later abandoned. The same author (1922) proposed to inject the 3 doses at 14-day intervals. The injections were made into the arm subcutaneously, the second dose being given in the opposite arm.

The local and constitutional disturbances varied considerably. On the whole, children who gave a plain positive Schick reaction showed no ill effect from the toxin-antitoxin injection, but the 'pseudo-combined' reactors often experienced local tenderness and swelling with constitutional disturbance, and temperatures up to $100-103^{\circ}$ F. for 1 or 2 days. In all cases, however, the reaction subsided without ill effects. It was noteworthy that the younger children seldom showed any harmful result; while adults, especially those giving 'pseudo-combined' Schick reactions, might have quite considerable local and general reactions, which, according to Park (1922) were rather less unpleasant than anti-typhoid inoculations.

O'Brien, Eagleton, Okell and Baxter (1923) reporting on nearly 600 cases observed nothing beyond trivial local reactions.

Zingher (1921) made the following suggestions for application in New York City:

"(i). Infants under 6 months are unsuitable for immunization owing to the frequent presence in their blood of antitoxin passively transmitted by the mother.

"(ii). Children between 6 months and 5 years should

"all be immunized without a Schick test. years).

"(iii). Children of 5 years upon entering school
"and coming under the influence of health officers
"for the first time should all be immunized without
"a Schick test, since most of them are susceptible.

"(iv). Older children up to 15 years should only be
"immunized if the Schick reaction is positive, since
"in some schools the number of susceptibles is small
"and the immunes are likely to suffer severer non-
"specific reactions after injection. ,000.

"(v). Children over 15 years, for the same reason,
"should only be immunized if the Schick reaction is
"positive. on in children under 10 years of age.

"(vi). Diplo "If the Schick test, carried out 6 months
"after immunization, is still positive, 1 or 2 fur-
"ther injections should be given and are usually
"sufficient to bring about the desired result."

"obtained. In Scotland these methods of prophylaxis
have received wider application relatively to the
size of the population than in England; in Edinburgh
for example, they have been used in 35 schools and
13 institutions, with re-visits in the majority of
cases. "The average numbers of children overtaken
"per month (5 weekly visits) at schools are 400
"school children (5 to 10 years) and 100 under
"school age children; and at child welfare centres

"40 pre-school infants (6 months up to 5 years).

"The test is omitted in the case of children under 5 years of age. It is safe to say that 90 per cent. of the children are completely immunized within 9 months of the completion of their immunizing course. In particular the answers to queries were as follows:-

"(i). Number of persons Schick tested 12,000.

"(ii). No ill-effects from testing.

"(iii). Number of persons immunized 8,000.

"(iv). Apart from a very few cases of stiffness and slight tenderness of the arm, no ill-effects from immunization in children under 10 years of age.

"(v). Diphtheria occurred in a comparatively small number of children who received protective injections but within the period which it takes for protection to develop and before negative Schick tests were obtained. The disease also occurred in a slight or moderate form in one or two isolated cases which had been immunized and rendered Schick negative, but in which there was some reason to believe that the operator's testing technique had not been above reproach and that the final tests had been mis-read or faultily recorded.

"(vi). Systematic immunization of the nursing staff

"at the City Fever Hospital was instituted in 1922 and
 "is being continued with highly satisfactory results.
 "The following table gives the incidence of diphtheria
 "among the nursing staff for the past seven years:-

Years.	Total Nursing Staff.	No. of cases of Diphtheria.
1920	148	10 = 6.75 per cent.
1921	146	14 = 9.58 " "
1922	147	13 = 8.84 " "
1923	137	5 = 3.65 " "
1924	128	4 = 3.12 " "
1925	161	5 = 3.10 " "
1926	153	2 = 1.30 " "

"It should be added that the cases of diphtheria oc-
 "curred in nurses who were not fully protected. For
 "instance, in 1925, of the 5 nurses who contracted
 "the disease 1 proved susceptible, but had not been
 "immunized; 3 developed diphtheria within six weeks
 "of the completion of the immunizing course, and 1
 "developed the disease in a very mild form one year
 "after immunization. In 1926, the 2 nurses who con-
 "tracted diphtheria suffered from a mild attack of the
 "disease six weeks and three months respectively after
 "completing the course." (Dr. Robertson M.O.H. City
 of Edinburgh, 3rd December, 1927. The Medical Officer

p. 249)). By results in America points to an effective

protection. The general conclusions on Schick testing and immunization against diphtheria (Eighth Annual Report of the Scottish Board of Health, 1926, p.19) are as follows:- after six and a half years the

great majority "Schick testing has been carried out on a considerable scale in Scotland, and also immunization with toxin-antitoxin and with toxoid-antitoxin. No untoward effect has been noted. In a number of cases diphtheria has developed at varying intervals after a course of immunizing injections, but most of these cases arose within a few weeks or months of the last immunizing injection, and probably before there had been sufficient time for the body to produce the necessary amount of antitoxin. A number of years will have to elapse before any opinion can be formed as to the efficacy of the methods of immunization employed against diphtheria. The results reported from large centres of population in America are in favour of the procedure, and there is reason to expect that results in Scotland will prove equally satisfactory."

even when The duration of immunity worked by T.A.T. inoculation can only be determined in course of time and by further experience. The evidence, so far

provided by results in America points to an effective protection lasting for certainly more than six years. Park, Schroder, and Zingher stated in May 1922 that 10,000 children had been under observation for three to six years, and after six and a half years the great majority of those who had developed antitoxic immunity still retained it. Later experience has shown that this period reaches ten years and thus tides over the most susceptible times of life, merging into the naturally acquired immunity of adult years.

Zingher (1921) showed that in children without antitoxin in the blood, the disappearance of the positive Schick reaction is slow, and sometimes feeble as judged by the fact that some cases do not become negative at all. He found in very large numbers of susceptible children that 2 months after the 3 T.A.T. injections only 30 per cent. were negative at the re-Schick, after $2\frac{1}{2}$ months 51 per cent., while after 5 months 87 per cent. were negative.

Zingher (1922) showed that a rapid response occurs in members of unusually immune populations even when they have no antitoxin in the blood according to the Schick test. To give one example: in a school in which only 33 per cent. of the children

were immune, the susceptibles were immunized and 5 months later only 25 per cent. of these susceptibles had become immune. On the other hand, in another school with originally 54 per cent. of immunes, the same immunization produced immunity in 41 per cent. of the susceptibles. This result is no doubt due to a greater degree of active immunity even among the Schick positive reactors of the second school.

O'Brien, Eagleton, Okell, and Baxter's (1923) results also bear out this effect of active immunity. In an institution with 62 per cent. of immunes, 83 per cent. of the susceptibles became immune 11 weeks after immunization. On the other hand, in another with, originally, 72 per cent. of immunes 91 per cent. of the susceptibles became immune in the same time. Zingher (1922) has observed that when the children who failed to react to the first doses are re-injected after 6 months, they then react as satisfactorily as the original successes. It should be realised that results purporting to be due solely to toxin-antitoxin injections may not be so due, but to a greater or less extent to a coincident natural immunization. For instance, in a school in which the basal immunity is due to the natural process following infection, the success of

T.A.T. injections must also be due, in part, to the natural process still operating. It is not, of course, possible to disentangle those cases which owe their immunity to a continuation of the natural process from those in which the natural process is responsible for the basal immunity and the artificial for the subsequent increase, but Dudley's (1922) work shows clearly that the natural process is capable, alone, in a suitable social environment, of producing an extensive immunity in a short time.

"H.L. Eder (Minnesota Med. October 27th 1927, p.584) advocates Larson's method of detoxifying "certain toxins with a low percentage of sodium ricinoleate. Such a soap toxin gives no reaction, is "stable under all conditions, contains no horse serum, and makes it possible to immunize against more "than one disease with the same common vehicle. "The combined soap toxin used during the past twelve "months (1926-27) was a 3 per cent. solution of "sodium ricinoleate containing 1000 million Scarlet "Fever streptococci and their toxins, and 0.1L + "diphtheria toxin to each cubic centimetre; 1 c.cm "of this solution is given subcutaneously over the "biceps, and repeated at weekly intervals for three "doses. In Minneapolis, where some 13,000 school

"children were so protected, statistics over a six
 "months' period show that the incidence of Scarlet
 "Fever among the uninoculated was 1 in 54, while
 "among the inoculated it was only 1 in 520. Of the
 "660 cases of diphtheria during the same period,
 "only 2 occurred among the inoculated children"
 (B.J.M. Oct.29th, 1927, p.65).

Okell, Eagleton and O'Brien ('Lancet',
 1924, i, p.800-801) have devised the following meth-
 od for the rapid control of diphtheria epidemics in
 institutions:-

- (i). Schick test all persons in the institution.
- (ii). If possible swab all persons, throat and nose.
- (iii). In twenty-four to forty-eight hours, isolate
 all Schick negative reactors.
- (iv). For a few days see twice daily all positive
 reactors (to detect any who may develop the disease).
- (v). Test for virulence all positive swabs from
 Schick-negative reactors, release "avirulent", but
 rigidly isolate all "virulent carriers".
- (vi). Begin at once to immunize all Schick-positive
 reactors.

These authors state that this method de-
 pends on the following propositions:-

- (i). Schick-negative reactors are not susceptible to

diphtheria.

(ii). Schick-positive reactors are susceptible to diphtheria.

(iii). Schick-positive reactors never harbour virulent bacilli detectable by ordinary swab culture methods unless they are suffering from or are incubating the disease.

(iv). Carriers of virulent bacilli are immune and are always Schick-negative reactors.

(v). "Avirulent" bacilli do not cause diphtheria, and therefore "avirulent" carriers are of no importance to the public health authorities.

(vi). One efficient swabbing of the population gives a sufficient working knowledge of the location of infection - that is, all profuse, and therefore dangerous, carriers will be detected.

Harries ('Lancet' 1924, *i*, p.921) worked out a somewhat similar scheme for dealing with diphtheria outbreaks in Scarlet wards of his hospital. He, however, omitted the virulence test, and thus instead of 6 classes, he divided the patients up into 4 classes.

It might be mentioned here that the 6 classes are as follows:-

- A Schick+, swab-negative.
- B " " , " + Bacilli avirulent.
- C " " , " " " virulent.
- D " Negative, swab-negative.
- E " " , " positive Bacilli avirulent
- F " " , " " " virulent.

Harries gives the Schick-positive, swab-positive, reactors prophylactic injections of diphtheria anti-toxin. In the case of adults who has still some time to spend in hospital before discharge, Harries gives 3 T.A.T. injections at weekly intervals of 1 c.cm. each.

In concluding this section, I must point out the necessity for keeping very careful records of all children Schicked and immunized, and for this purpose each child tested requires a history card on which are recorded the following details:-

- (i). Name, age and school.
- (ii). Personal history of previous diphtheria, scarlet fever, asthma, hay fever, nettle rash.
- (iii). Family history of Scarlet Fever, Diphtheria, etc.
- (iv). Date of Schick Test and readings thereof.
- (v). Date of T.A.T. injections and dosage.
- (vi). The date and result of re-Schicking.

(vii). If still a positive-Schick reactor at the re-Schick, the date and number of subsequent T.A.T. injections.

(viii). The results of final Schicking.

(ix). Bacteriological findings, if any.

SECTION IV.

Treatment.

(b) Curative.

Beal Schick (1923) states that the dose of antitoxic serum is to be computed according to the weight of the body, and that the injection should be made at the earliest possible moment. "In all mild and medium cases 100 antitoxin units per kilogram of body weight are sufficient. In severe cases 500 antitoxin units per kilogram of body weight should be given. Repeated injections should be omitted as superfluous. Fifty antitoxin units per kilogram of body weight suffice for the purpose of immunization. In dangerous cases the antitoxin should be given intravenously as there is a slight improvement in the curative results" Schick (Boston, Med. Surg. Jour. 1923, cl.xxxviii, p.255-256).

The introduction of antitoxic serum by von behring has revolutionised the treatment of diphtheria, and whereas treatment in pre-antitoxic days

was practically useless, it is now extremely effective and if we could be certain of seeing our patients on the first day of their illness very few words would be needed to describe it. It would be sufficient to say that antitoxin should be given at the earliest possible moment. Kolmer gives some very interesting statistics from Philadelphia experience, comprising some thirteen thousand cases over the period from 1904 to 1913. The fatality rates corresponding to the day of the disease on which antitoxin was begun are as follows:-

First day.....	0.4	per cent.
Second day.....	5	"
Third day.....	8.3	"
Fourth day.....	10.7	"
Fifth day.....	11.2	"
Sixth day.....	14.2	"
After sixth day.....	13.1	"

Patients often come comparatively late under treatment, or again, the true nature of the disease may not at first be fully realised. And so it is that diphtheria still remains a most dangerous infection, and one for which some other forms of treatment besides antitoxic serum administration are necessary.

Preparation of Antitoxin

A. Preparation of the Diphtheria Toxin.

The first necessity for the production of antitoxic serum is the preparation of a strong toxin, for example, P8 is an organism isolated by Park and is the one form from which the toxin is prepared as it yields the strongest toxin.

The organism is grown on broth for 7 days, a surface growth being aimed at. Filtered through a Berkfeld filter. The filtrate is the crude toxin free from organisms.

B. Standardising of the Toxin.

A guinea-pig of 250 grammes weight is used. The least amount of toxin that will kill the standard guinea-pig in 5 days is the standard of toxin used. It is known as M.L.D. (Minimum Lethal Dose).

C. Immunization of the Horse.

Subcutaneous injections of the toxin are given to the horse. Commencing with 1 c.cm. of weak toxin which contains 50-100 M.L.D. Injections are given at intervals of 3-4 days, the dose increasing slowly at first but more rapidly afterwards, depending on the local swelling, temperature and general condition of the horse. At the end of 6 weeks or so a sample of the horse's serum is drawn

off and tested for strength.

D. Mode of obtaining the Serum.

When the sample of serum tested is thought of sufficient strength, the horse is bled to the extent of 8-9 litres. The blood is collected in vessels containing Potassium Oxalate in such proportions that the final strength after blood is added shall be 1 in 1000. Red Corpuscles settle down in a day or two. Plasma is siphoned off. Sufficient Calcium Chloride is added to precipitate all the oxalate and leave a little in excess, and 0.3 per cent. trik-resol or other antiseptic is also added as an additional safeguard against contamination (aseptic precautions are taken throughout the preparation of the antitoxin). After coagulation is complete the serum is siphoned off.

E. Standardisation of the Serum.

The serum is tested for unit value - it is found to vary from about 400-1700 units per c.cm. The sera of various strengths are mixed to get one of average strength (450 units). The mixture is again filtered. The mixture again tested for unit value and also for sterility. If it satisfies all the tests it is run into phials and is ready for use.

The antitoxic unit is the smallest amount

of antitoxin which will neutralise one hundred times the minimum lethal dose (M.L.D.) given to a guinea-pig of 250 grammes weight.

An average commercial serum contains about 500 units to the cubic centimetre, but some serums are of the strength of 1000 units to the cubic centimetre, and naturally these concentrated serums are more expensive to produce, and their cost is correspondingly higher. The antitoxin is often guaranteed to preserve its labelled strength for at least a year.

Mode of Administration.

The subcutaneous route originally employed is still probably that used by the vast majority of practitioners. The injection may be made in the flank or into the loose tissue between the shoulder blades. The usual aseptic precautions are taken and the puncture sealed with collodion and cotton-wool. The subcutaneous method is distinctly inferior to the other methods of (a) intramuscular and (b) intravenous.

The intramuscular injection is given into the substance of vastus lateralis, the needle being inserted on the outer aspect of the thigh. This method is much less painful and can usually be given

more quickly than the subcutaneous route.

The most effective route, however, is undoubtedly that of intravenous injection. Dose for dose, when 2 units per c.cm. of blood are obtained in six hours by the subcutaneous method, 20 units per c.cm. are found present if the intravenous route has been used. Park, who gives these figures, also considers that antitoxin is absorbed twice as rapidly when given intramuscularly instead of subcutaneously. He adds that whereas there is a slow increase of antitoxin with the latter method, there is a steady decrease with the intravenous method, though even after twenty-four hours there is a difference in favour of intravenous injection, 12 units per c.cm. as against 6 after subcutaneous. Ker recommended in very severe cases that an initial dose of 10,000 units should be given intravenously, followed in 8 hours by 8000 or 10,000 intramuscularly, a third dose being occasionally added. This would seem to secure a fairly high antitoxin content in the blood for some time. In all cases in which intravenous injection is used the serum should be warmed to approximately blood heat.

The administration of serum either by the rectum or the mouth need not seriously be considered

as a therapeutic measure. *Thomson 1921.*

Antitoxin We have to consider (a) how much antitoxin should be given and (b) whether one dose or several doses are required. Park (1921) stated "that in no case is sufficient toxin elaborated to require more than 100 units to bind it completely. Immensely larger doses are properly given so as to reach the free toxin everywhere quickly, and in adequate amount."

Two useful tables of dosage are given by very experienced authorities, thus:-

Park 1921.

Antitoxin Units to be given in the Various Types of Cases.

	Mild Cases.	Early Moderate.	Late moderate and early severe	Severe & Malignant.
Infants 10-30lb in weight; under 2 years.	2000-3000	3000-5000	5000-10,000	7500-10,000
Children 30-90lb in weight; under 15 yrs.	3000-4000	4000-10,000	10,000-15,000	10,000-20,000
Adults 90lb and over in weight.	3000-5000	5000-10,000	10,000-20,000	20,000-50,000
Method of administration : advised	Intra-muscular.	Intra-muscular.	Intra-venous.	Intra-venous.

thought necessary and Thomson 1921.

Antitoxin Units to be given in the Various Cases.

	<u>If treated</u>	<u>If untreated</u>		
	on 1st day	2nd day	3rd day	4th day or later
Mild faucial	6000	6000	8000	10,000
Moderate	10,000	12,000	14,000	16,000
Severe, severe faucial and laryngeal	12,000	24,000	30,000	30,000
Very severe faucial	30,000	40,000	50,000	50,000
Laryngeal only	18,000	18,000	18,000	18,000
Nasal only, but with membrane conjunctival only	6000	6000	8000	10,000

These tables may be taken as embodying a system of dosage which adequately represents modern scientific knowledge and clinical experience. The Danish physicians, however, give even larger doses e.g. in the Blegdams Hospital, Copenhagen, single doses of 100,000 units are given to the most severe cases, and as much as 340,000 units have been administered in a succession of doses to a single individual (Bie, 1922, Acta Medica Scandinavica, lvi, 337). Park believes (1921) that it is of fundamental importance to give at once the whole of the antitoxin that is

thought necessary and it is an error to split this quantity up into several doses. An insufficient first dose cannot be wholly compensated for by later injections. Other authorities, however, in Denmark and in Germany employ repeated doses (Bie, 1922; Friedemann, 1922). The last-named author considers that the repetition of large doses is an important factor in the cure of severe cases.

E.W. Goodall (1928, 'Injections Diseases', Third Edition, page 197) after a large experience concludes that any amount of serum over 30,000 units, even when spread over two or three days, is wasted, and that in the large majority of cases a smaller amount will suffice.

Ker (1921) gave 1500 units as the smallest dose and this to cases which were more bacteriological than clinical diphtheria. In a letter to the 'Lancet' (1923, pp.642-643) Ker with his vast experience pointed out that very large doses of serum had not secured any better results than can be obtained by more moderate dosage. Ker's largest total dose never exceeded 64,000 units. He found that in severe naso-pharyngeal and laryngeal cases it may be necessary to give in all 40,000 units or even more. In not a few cases there is no visible

improvement until the patient has received over 20,000 units, and there is no doubt that, when a diphtheritic broncho-pneumonia is supervening in a laryngeal case, much is to be gained by pushing the serum till the respirations and temperature fall. Except in this class of cases, a fall of temperature is not to be regarded as a sign to stop the treatment unless, indeed, the patient is much improved in other respects. The worst instances of diphtheria often run their course with the temperature normal or subnormal.

It is a matter of almost universal experience among physicians that the efficacy of anti-toxin is greater when it is given early in the attack, and that it decreases as the day of administration is delayed. "It is fatal to wait for the result of "cultures, except perhaps in the case of the most "trivial of throats. When once a case is suspected, "an injection should be given. The diagnosis can "be made afterwards. Even a small dose, for instance "1500 units, may be of the utmost value in checking "the spread of a membrane, and can do the patient no "harm whatever. A few hours may make an enormous "difference in the prognosis of any given case. I "have never seen a fatal result in a case which de-

"veloped in hospital, and in which injection was
"practised on the first day of the disease." (Ker,
1921).

Serum Sickness and Anaphylaxis.

A certain percentage of cases will present sequelae which are apparently due to the injection of a foreign protein. They occur, indeed after injections of normal horse serum and are in no way due to the antitoxic bodies contained in a therapeutic serum. They have been explained by von Pirquet and Schick as an anaphylactic phenomenon and, although some of us might prefer to reserve the term anaphylaxis to those cases in which a previous dose of serum has been injected, no doubt the process is fundamentally the same.

It may be assumed that antibodies to the foreign protein are produced, and that after an interval, which varies considerably but is often from 8-12 days, they have developed or accumulated sufficiently to interact with any of the horse serum which may remain in the body cells. The interaction produces an apotoxin which causes the symptoms of the serum disease. If, then, before the requisite development of the antibodies the foreign protein in the horse serum has all been got rid of, no

reaction occurs and no serum phenomena result.

The principal features of this serum sickness are firstly various forms of rashes, secondly pyrexia, and thirdly joint pains. These may occur separately or in combination. Of the less prominent manifestations may be mentioned slight oedema, affecting usually the loose tissues such as the eyelids or prepuce, but also occasionally seen in the hands and feet. A general puffiness of the face is not at all uncommon. Adenitis is also sometimes a symptom, the glands draining the site of injection being most distinctly affected. Occasionally the more usual manifestations are accompanied by some congestion, or even patching, of the fauces. This form of sore throat is most commonly observed in the more severe examples of the condition. Lastly it is important to recognise that vomiting may accompany or precede these different serum phenomena. It is not, however, at all common. The most obvious and important of the symptoms are undoubtedly the various forms of rash. While rashes of a mixed character are frequently observed, four main types are generally recognised.

1. Urticarial_rashes - These rashes are perhaps the most common of all. Large irregularly shaped

scattered blotches appear on the skin, the neighbourhood of the site of injection being usually affected first. In many cases true urticarial wheals rise in the centre of the blotch, and, if so, there is intense itching and irritation. Sometimes, however, while the appearance and distribution of the rash resembles urticaria, no wheals appear. The rash is often very profuse and fresh plaques may continue to come out for two or three days. Occasionally, on the other hand, only two or three blotches are noticed, and may remain visible for less than twelve hours. The most common time for this form of rash to appear is from the eighth to the eleventh day after injection.

2. Multiform erythema - This type of rash is also extremely common. Often it is circinate in its arrangement, perhaps more frequently it assumes the character of large patches mixed with a scattered and somewhat morbilliform erythema. Different parts of the body may present quite different appearances at the same moment. The face is almost invariably blotched and often puffy. In colour the rash is usually a somewhat bluish pink. It may change much in appearance from day to day, and sometimes lasts for over a week. Occasionally it

becomes haemorrhagic and the skin may be left deeply stained. This, however, is not at all usual. A mixed erythema often succeeds an urticarial eruption after two or three days' interval.

3. Morbilliform rashes are said to be common, but Ker had seen very few which resembled the rash of measles sufficiently closely to cause trouble in diagnosis. The relative frequency of the different types of rash probably depends to some extent on the particular serum used, which, no doubt, accounts for differences of opinion on this point. Sometimes the whole body, including the face, is covered with a uniform measly rash. As the conjunctivae are liable to be congested in almost any variety of serum sickness, and as puffiness of the eyelids is not at all uncommon, the resemblance to measles is, at times, sufficiently striking. But catarrh is conspicuous by its absence, and the buccal mucous membrane shows neither stomatitis nor Kopliks spots. There may, moreover, be a complete absence of fever. Even if it is present, the chart shows no prodromal rise of temperature. The rash, again, is not raised as much above the skin as is the case in measles, and, a point of much less diagnostic value, it often starts from the site of injection, and not on the

face. ~~ver,~~ or with such signs as headache, vomiting,

4. Scarlatiniform Rashes - It is, Ker stated, beyond all question that serum rashes are not infrequently of a scarlatiniform type. Some writers would regard all such rashes as instances of true scarlatina, but, while the possibility of this infection must always be carefully considered, it is only occasionally the cause of the rash. The skin may present merely a uniform erythematous flush, which only superficially resembles the Scarlet Fever exanthem, or, on the other hand, there may be definite punctation, in which case the skin appearances are absolutely indistinguishable from those of Scarlet Fever. Rashes of this type usually occur early, that is to say the majority of them seem to appear from from the second to the sixth day after injection. Ker had been accustomed to associate them with particular batches of antitoxin. Like the other varieties of serum rash, they may, or may not, be accompanied by pyrexia, and sometimes, though rarely, arthritis is present. The differential diagnosis of these rashes from scarlatina is one of the greatest troubles of fever hospital life. Ker lays most stress on the absence of constitutional symptoms. A rash, unassociated with the slightest suggestion

of fever, or with such signs as headache, vomiting, or shivering, is probably due to serum, however closely it resembles the scarlatinal exanthem. On the other hand, rises of temperature, even so moderate as 99° F., increase the possibility of the case being one of mild scarlatina. Fever, indeed, though perfectly compatible with a mere serum manifestation, should always arouse suspicion.

Even in cases with a marked febrile reaction, however, the presence of a single urticarial or erythematous blotch on the face, or elsewhere, points clearly towards a diagnosis of serum sickness, as would also the appearance of definite joint pains on the first day of the eruption, a date which is distinctly early for scarlatinal arthritis. Well-marked fever, without such signs would strongly predispose us to a diagnosis of scarlatina, and this view would be much strengthened if initial vomiting and sore throat have been noticed. Here again we are confronted with the difficulty that the throat is sometimes affected in serum sickness, and that vomiting, though rare, is occasionally met with. But the conjunction of symptoms so suspicious with a definite punctate rash, and more or less pyrexia, should justify a diagnosis of scarlatina. Two

other points should be mentioned. The condition of the tongue and the manner of its peeling should be closely watched - typical peeling will indicate Scarlet Fever even though the temperature has not been elevated. Desquamation, even in serum rashes, may resemble that of scarlatina, but it is much less definite and usually does not involve the palms and soles.

The rashes due to serum may appear at almost any moment within four weeks from the day of injection, but the period between the seventh and eleventh days, inclusive, seems to be the most usual time of occurrence. Ker has seen blotchy rashes as early as twelve hours after injection, but it is seldom that a rash is seen before the third, or after the eighteenth day. Joint pains are most frequently met with, either with or without rash, from the tenth to the twelfth days. ¹"Repeated injections of certain substances in sub-toxic or non-toxic doses - a suitable interval of time elapsing between the injections - may be followed by markedly toxic or even fatal symptoms." It is to be noted that this definition refers to observations made on laboratory animals, and that the central feature of the process is the occurrence of symptoms after a second injection.

¹ Amaphylaxis., Muir & Ritchie, 1919 define anaphylaxis as follows:-

tion, when a certain interval has elapsed between it and the first. The question therefore arises, to what extent the collapse after injections of anti-toxin is covered by this definition. Ker stated that there are perhaps, fifty or fewer examples in the literature of sudden death following serum injection, and the accident seems most usually to have occurred in children suffering from status lymphaticus or persons who have been subject to asthma. In the less severe cases of shock which end in recovery and which have been more frequently observed, the patient is collapsed and suffers from distressing dyspnoea. The face becomes cyanosed and oedema appears rapidly. The pulse is soft, small, and rapid. Usually a rash, most frequently urticarial, sometimes scarlatiniform, develops within half-an-hour. It may, indeed, be observed much earlier. Rashes seen in these immediate, as also in the accelerated, reactions are usually very profuse and extremely irritating, and the discomfort of the patient may be extreme. Dangerous anaphylactic symptoms are very rare in man. Richet (1916) stated that these accidents in man are extremely rare, amounting to not more than 1 in 300,000. They have been reported as occurring at least as frequently after a first

injection of antitoxin as after a second injection. (Netter, 1916).

They have usually occurred in sick persons who are already liable to sudden heart-failure. Many hundreds of thousands of men were injected more than once during the war with antitetanic horse serum without showing acute symptoms. Moreover, a number of the fatalities that have been reported as cases of 'anaphylactic shock' are open to considerable criticism. For example, the case quoted by Gurd and Emrys-Roberts (1920) was a first injection, and in H.R. Dean's case (1922) the shock only occurred after the 4th dose, although the patient was already fully sensitised before the third. Reviewing the facts, the authors of the Medical Research Council (Diphtheria, 1923) conclude that the proof is lacking that these occurrences are of the same nature as the experimental anaphylactic shock in animals. The same conclusion applies even more clearly to the ordinary serum sickness, which is widely believed to be of anaphylactic origin (Richet, 1911; Muir and Ritchie, 1919). Ker advised desensitisation of asthmatic subjects and all persons who are known to have had injections of serum within three years. It is customary to attempt desensiti-

sation by the injection of a minute quantity of serum, 0.5 c.cm, subcutaneously, and then give the therapeutic dose four hours later.

Practical considerations regarding the Accidents of Serum-treatment.

(i). The concentration of antitoxin, which facilitates the injection of a smaller quantity of serum-solids per unit of antitoxin, is the most promising means of reducing the incidence of serum-sickness. Caution must, however, be exercised in this matter, owing to the uncertainty whether the serum may not be losing some of its healing properties in the process.

(ii). Serum-sickness is best considered as an instance of 'allergy', a form of hypersensitiveness closely allied to, but not identical with, anaphylaxis. It is doubtful whether true anaphylactic shock ever occurs in man.

(iii). Serious accidents after inoculation or reinoculation with antitoxin are so rare that no physician ought ever to be driven by the fear of them to withhold antitoxin in any case of diphtheria.

(iv). Both shock and severe serum-sickness tend to be somewhat more frequent (a) when the individual has had one or more previous injections, and (b) when

the antitoxin is given intravenously. Intravenous reinjections should therefore be practised with considerable caution, and reserved for the gravest and most urgent cases.

Local Treatment.

This takes a very secondary position when compared with the serum treatment and general management of a case. It is nevertheless well to keep the mouth and throat as clean as possible by frequent swabbing, and for this purpose the following prescription was recommended by Ker:-

Toluol	36 parts	} To 100 c.cms. of this solution 10 grammes of menthol may be added.
Absolute alcohol	60 "	
Tinct. ferri. perchlor.	4 "	

A pledget of cottonwool, impregnated with this solution, is pressed firmly upon the false membrane, care being taken to avoid swabbing the unaffected parts of the throat. The toluol must not be used too frequently, thrice daily being quite sufficient. To assist in keeping the fauces relatively clean, and to remove loose shreds of membrane, boroglyceride and glycerine in equal parts may be used on a swab, at intervals of about 4 hours. Those patients able to gargle may do so with listerine, a dessert-

spoonful to the tumbler of hot water, or with chlorine water, which, if not so pleasant, is said to be extremely effective. Sprays of peroxide of hydrogen, or corrosive sublimate in a 1-4000 solution may also be freely used both for the throat and nose.

Douching a syringing may often be of great advantage, and for adults with much faucial oedema steam inhalations with or without tincture of benzoin or creasote are very comforting. Many children resist local treatment and are perhaps best left alone.

In patients who have shown any tendency to haemorrhage the throat and nose may be sprayed with a solution of adrenalin chloride.

Should the glands be much enlarged and form a collar round the neck, fomentations wrung out of weak carbolic lotion (1-60 to 1-80), may be frequently applied, or the neck may merely be wrapped up in cotton wool.

General Management:-

Having given the patient an adequate amount of anti-toxin, it is very important to keep him in the recumbent position for about three weeks, or longer if the condition of the heart indicates it. From the moment diphtheria is diagnosed the patient must be kept flat, and forbidden the slightest exertion.

He is on no account to be allowed to sit up. One soft pillow is sufficient, as it is desirable to keep the head low. Active movements, such a reaching to the bedside table for toys or books, must be strictly forbidden. The bowels should be carefully regulated to avoid straining at stool. unnecessary to modify

the diet Reading should not be indulged in for a fortnight, even in mild adult cases. It is undoubtedly the case that undue exercise of any function is apt to encourage paresis, and this is especially true of accommodation and oculomotor paralysis. pharyngeal

paralysis After a fortnight, when the lesions have been trifling, adults may be allowed to read for half an hour or so at stated times. Towards the end of the third week, provided the pulse is satisfactory, a second pillow may be allowed, and thereafter the patient may be gradually propped up, and perhaps be allowed out of bed by the end of the fourth week. In severe cases, however, or moderate cases followed by pulse irregularity, it may be necessary to keep the patient absolutely recumbent for six or seven weeks. The nurse should take pulse and temperature every four hours, and report at once any marked change in the pulse rate, any vomiting, any complaint of faintness or pain, or any paralytic symptom which

comes under her notice.

The diet should at first be fluid and as liberal as possible. Milk, custard, strong beef tea, and so forth, should be given. When all fever has subsided, soft solids and in mild cases an ordinary diet may be allowed. It is unnecessary to modify the diet on account of albuminuria. The object should be to maintain the strength by suitable feeding. In cases of tracheotomy or intubation the nasal tube will be required, and it should also be used at once if there is any suggestion of pharyngeal paralysis.

Rectal feeding must be resorted to if there is persistent vomiting.

The diphtheria patient takes stimulants well and is usually the better for them. It is wise to give very small amounts of whisky at regular intervals as a routine, except in the mildest cases.

If there is any tendency to vomiting, brandy is tolerated better, and champagne may be tried for cardiac sickness.

A very secondary part is played by drugs in the treatment of diphtheria. Strychnine given systematically is useful, but it cannot be said either to avert cardiac syncope or to be of any use

be applied in the intervals.

when the latter has occurred. It, probably, is of real value in the treatment of paralysis. Iron is to be recommended in some form or other during convalescence, and some preparation of the nature of Easton's or Fellowes' syrup may be used with advantage. (Ker 1921).

Nasal diphtheria:- is treated by frequently syringing or irrigating the nasal passages with an alkaline solution such as the following:-

Sacchar alb.

3j

Sod. Biborat.

3j

Sod. Bicarb.

3j

Potas. Chlorat.

3ss.

Sod. Chlorid.

3ss.

Tinct. Lavand. Comp.

3j.

Aq. ad.

0j.

Constant attention is required to keep the nostrils from becoming ulcerated. For epistaxis the injection of iced water, or the external application of ice is recommended. As mentioned above adrenalin chloride may be sprayed up the nostrils.

Diphtheria of the vulva, conjunctivae, and wounds is best treated by frequent washings with antiseptic solutions; while hot boracic fomentations should be applied in the intervals.

In paralysis or paresis of the respiratory and pharyngeal muscles, the foot of the bed should be raised as saliva tends to flow out of the mouth rather than to collect over the glottis. Paralysis is best treated by complete rest of function and the internal administration of strychnine. Electrical treatment is of no use. The nasal tube should be always employed for feeding purposes. Antitoxin should not be given, firstly because it does no good, secondly because the patient having been sensitized to it, a second dose after a long interval may cause unduly severe serum sequelae.

Treatment of Heart Failure:- When cardiac failure occurs there is unfortunately but little to be done. In sudden collapse hot fomentations may be applied over the heart, and ether or strychnine injected hypodermically. The foot of the bed should be raised on blocks and the pillow removed. The injection of normal saline either subcutaneously or into the rectum is always worth trying. The feeding should be rectal, though iced brandy or champagne is often tolerated by the stomach.

Treatment of Laryngeal Diphtheria:-

The great object at first will be to avoid operation if possible. Everything should be done to palliate the condition and to give time for the serum, which should be given immediately, to exercise its action. The breathing is usually much relieved by the steam kettle, and the application of hot fomentations to the throat is often useful. In the Edinburgh City Hospital steam is laid on from the boilers and supplied to the patient through pipes on swinging brackets at each side of the cot at a pressure reduced to about $3\frac{1}{2}$ pounds - this is an extremely efficient method. In asthenic cases on the first day of illness the old plan of freely administering ipecacuanha wine has its advantages, but as the disease progresses the condition of the heart is not likely to encourage an emetic treatment. Belladonna pushed freely is of considerable value in relaxing spasm. The patient nearly always requires alcoholic stimulation.

Indications for operative interference - In private practice, where the medical man cannot be in constant attendance, it is obviously wise to operate early. Should dyspnoea be present with

definite recession of the soft parts of the chest, it is difficult to see how the patient can be safely left unless tracheotomy is performed. If the child cannot be sent to hospital, the best course is to operate at once.

In hospital practice, on the other hand, it is well to wait as long as possible, and the condition of the pulse is in the long-run the safest guide. Provided a medical man is always within reach, the patient may be allowed to enter the third stage of croup. It is also advisable to consider the time the patient has been ill. If there is reason to believe that the toxins have had time to affect the cardiac muscle, we would naturally not postpone operation too long for fear of death from shock on the table.

Tracheotomy and Intubation:- In this country intubation is not regarded as a suitable operation to employ in private practice, although in some hospitals, including the Edinburgh City Hospital, it is practised as the operation of election. It consists of the introduction, by means of a special instrument, of a vulcanite tube into the larynx. The tube is retained in position by its own weight, by its shape which expands below the constriction which lies in the glottis, and by its

rounded head which is supported by the aryteno-epiglottic folds of mucous membrane. It may be worn for as long as three or four weeks without injury to the larynx, but in most cases can be removed safely within three days. From its liability to be coughed out, and from the fact that a nurse cannot be expected to be able to replace it, its use is contraindicated, unless a doctor is always ready to reintubate if required. In hospital practice, however it should certainly be tried first. If it fails to relieve the obstruction, tracheotomy can be performed, and the patient is no worse off. The advantages of intubation over tracheotomy are : that it does not require anaesthetic; that it is performed in a much shorter time; that it necessitates no open wound, and therefore no scar (and the wound of tracheotomy is the source of various troubles); and that it enables the patient to breathe through the natural passages. During the ten years 1905 to 1914 there were 586 cases of laryngeal diphtheria at the Eastern Hospital which were tracheotomised or intubated; in 295 cases intubation only was performed, and 28 died, a fatality of 9.4 per cent.; in 159 cases there was

first intubation and then tracheotomy, and 74 died, a fatality of 46.5 per cent.; in 132 tracheotomy only was performed, and 54 died, a fatality of 40.9 per cent. The fatality of all the cases taken together was 26.6 per cent. (Goodall 1928).

The moment to be chosen for operative interference in croup complicating diphtheria varies much in different cases and in different circumstances. If the dyspnoea is urgent, the patient restless and seriously distressed, or there is marked recession, an operation should be performed at once. But there are cases in which, although the larynx is clearly involved, and even membrane may be coughed up, yet the dyspnoea does not at any time become urgent. Such symptoms of laryngeal implication may continue for some days, and the patient ultimately recovers without operative interference.

It would be a mistake in hospital practice in such cases immediately to perform tracheotomy or intubation, though there is less objection to early intubation than to early tracheotomy. We say in hospital practice, because there everything is ready should occasion arise - both operator and instruments. But in private practice it is a different matter. Slight symptoms are liable to become severe

at any moment; and it is wise to decide on tracheotomy as soon as the larynx shows signs of becoming seriously affected. In all cases of intubation or tracheotomy it is desirable to have the service of skilled nurses, in order to carry out efficiently the all-important after-treatment.

Intubation:- should not be performed unless the instruments for tracheotomy are at hand.

Occasionally though extremely rarely, membrane may be rolled up in front of the entering tube completely plugging the trachea. The patient should be pinned firmly in a blanket and placed in the recumbent position. There is no justification for adopting the sitting position in a disease like diphtheria. The gag is then inserted on the left side of the mouth, and the operator, standing on the right of the cot and well above the patient, introduces the left forefinger into the mouth and finds the opening of the glottis. To do this the epiglottis may have to be hooked up. The top of the finger should rest on the arytenoid cartilages. These, however, cannot always readily be recognized, and the best rule is to find the opening of the larynx and slide the finger back to its posterior margin.. The tube is then passed along the side of

the forefinger till it reaches the tip, when its point may be slid beneath it and thus rests directly over the glottis. Taking the utmost care to keep the instrument in the middle line, the operator then proceeds to raise the handle of the introducer, tilting the tip of the tube into the opening of the glottis. Half the secret of intubation is to remember to raise the handle sufficiently, as the impression given in performing the operation is that the opening of the glottis looks almost directly backwards.

Then, allowing the tube largely by the weight of the instrument to fall into the larynx, and taking care to exercise no undue force, the operator sets it free of the obturator by pressing forward the cylindrical arm, and follows the head of the tube with his forefinger, pushing it firmly home, when it is at once partially covered by folds of mucous membrane. Occasionally a spasm of the glottis resists the entrance of the tube, in which case, always provided the middle line is carefully maintained, a little gentle pressure may be exercised. A string must always be looped through the hole in the collar of the tube, so that, if it falls into

the oesophagus, it can be withdrawn. This can be removed by pulling on one end of the loop, through Ker preferred, to leave it in position attached by a strip of plaster to the cheek. If this is done, it is well to use a string which cannot easily be bitten through, and banjo string (No. 6) will be found excellent for the purpose. It should not, however, be looped through the hole, but attached as a single string with a knot on the end, which prevents it slipping through. It should be drawn taut, as otherwise a child, twisting his tongue round it, can extubate himself. The advantage of leaving the string in position is that a nurse can pull out the tube at once, should it become plugged, or should loose membrane, collecting below it, impede the breathing.

Again extubation, with the instrument provided, is much more difficult than intubation. The short French tubes can be 'enucleated,' or pressed out, which is easy in the sitting position though more difficult if the patient is kept recumbent. The method consists in pressing firmly on the trochea, just below the larynx, with the thumb, while the other hand placed firmly below the occiput simultaneously flexes the patient's head on his chest. This squeezes the tube out of position and the patient is instructed to spit it out.

The string is, on the whole a handier method, and is, if fixed carefully, no trouble to the patient. On the other hand, it necessitates the arms being controlled in cardboard splints, as otherwise the patient would pull the tube out. The length of time during which the tube should be worn is a matter of some interest. Some remove it for the first time in twenty-four hours, others leave it in position for as long as six days. The shorter period would be, doubtless, quite suitable for cases in which the intubation is a precautionary rather than a therapeutic measure. The psychological moment for removing the tube is from two and a half to three days from its insertion, and, always provided the temperature and respirations are normal, there is then a fair chance that the patient will escape further manipulation. If, however, there is still pyrexia and the breathing is rapid the tube will often have to be replaced, sometimes in a few minutes, sometimes after a couple of hours. It is rare for a patient who has breathed satisfactorily for as long as four hours to require a second intubation. Should, however, the tube be reinserted, an attempt should be made to remove it every second day. The greatest number of reintubations which Ker has performed

in any successful case is fourteen, and the patient in question, after wearing a tube for four weeks, recovered satisfactorily, with no sign of ulceration of the larynx. There is little advantage to be gained from a secondary tracheotomy undertaken to dispense with an intubation tube. It is better to persist with the latter, especially as light vulcanite tubes can be worn without damage for long periods. Ker stated that out of 268 consecutive cases five examples of laryngeal stenosis occurred, a proportion of 1.8 per cent. It is interesting that two of these cases were intubated for causes other than diphtheria.

Ker concluded that tracheotomy gave better results in the hands of the novice than intubation, and that statistics were of little value in estimating the value of the rival methods.

Tracheotomy:- This is the operation which should be selected by the general practitioner. If the symptoms are not too urgent, the skin of the throat and upper part of the chest should be carefully cleansed and prepared. Unless the patient is moribund, an anaesthetic is advisable, and if the patient has not been ill for more than three or four days he will probably stand chloroform well.

Should, however, the illness have lasted longer, eucaïne may be used with advantage as a local anaesthetic. The child should be immobilized by a blanket wound round the limbs and trunk up to the level of the nipple. The head should be held firmly by an assistant, the back of the neck resting on a rolled sheet or sand-bag of about 6 inches in diameter. The operator standing on the right of the patient satisfies himself as to the position of the cricoid cartilage, and, having discovered this anatomical point, is careful not to lose it. Steadying the larynx between the thumb, and middle finger of the left hand, with the forefinger resting on the lower margin of the cricoid, he then makes his skin incision downwards from the tip of the finger, being careful to keep the middle line. About an inch and a half is the usual length of incision required. Once the skin is divided, if the patient appears in extremis, a bold plunge into the trachea is quite justifiable, the left forefinger being shifted into the wound and still marking the lower margin of the cricoid. When time is no particular object it is well to dissect more slowly down to the trachea, not paying too much attention to hæmorrhage, which can be trusted to stop when the dyspnoea is relieved.

The covering aponeurosis of the trachea must be thoroughly divided and the trachea itself opened by an incision through the first two or three rings. The wound is then held open by dilators or by the handle of the knife held crosswise. No attempt should be made to insert the tube until the breathing is easy. Should any loose membrane be visible, an attempt may be made to remove it with laryngeal forceps: A little manipulation is sometimes required to introduce the tube, but its lower end once in the opening it is easily manoeuvred into position and tied by tapes passed through the openings in the shield and round the neck. A light gauze dressing covered with gutta-percha tissue is arranged round the shield.

A steam tent is not desirable in the after treatment of a case, but the air may with advantage be kept warm and moist by a croup kettle near the bed. The inner tube must be removed and cleaned occasionally. The wound may be dressed and the gauze changed twice a day. The feeding should be carried out entirely by the nasal tube. An attempt should be made, if the circumstances are favourable, to remove the tube in three days from the operation. The breathing can be tested by the removal of the inner tube and the plugging of the orifice, and if

satisfactory the tube can be dispensed with. Some patients, however, wear a tracheotomy tube for weeks, the breathing being obstructed by granulations above or below the wound. Nervousness is also apt to hinder the removal. Goodall recommends, in the latter case, that a dummy tube should be inserted when the removal of the original tube causes unsatisfactory breathing.

Broncho-pneumonia is a much dreaded and fatal complication of diphtheria, and especially of cases of laryngeal diphtheria where tracheotomy has been performed. It depends in most instances on septic infection, and streptococci are probably most often responsible. Occasionally, however, we have reason to believe that the process is purely diphtheritic in nature. The symptoms are an elevated and persistent temperature, accompanied by rapid breathing and some cough - the physical signs may be obscured by the noise of respiration through an intubation tube or a tracheotomy canula, but fine crepitations may be detected.

Otitis media is an occasional complication of diphtheria in convalescence, and the treatment is on the usual lines.

Labial Herpes - according to Rolleston may be expect-

ed in about 4 per cent. of diphtheria patients, and usually occurs early at the time of the faucial lesion. Nephritis is a very rare complication and endocarditis is most unusual.

Relapse:- Slight recrudescences of the throat condition are not uncommon, but a serious relapse during convalescence is comparatively rare.

Membrane again appears in the fauces and the disease is repeated. It is, however, unusual for such cases to be fatal. Both recrudescences and relapses occur most frequently about three or four weeks after the original attack. Some patched throats appearing at this period seem to be serum phenomena, and are not really diphtheritic. Second attacks of diphtheria are common.

In 23 years experience at the Edinburgh City Hospital Ker reported (1921) that he had had many patients under his care more than once, and in one case both attacks were of more than average severity, although the interval between them was less than two years.

Summary and Conclusions.

(1) The Schick test is an accurate indication of susceptibility or non-susceptibility of an individual to diphtheria.

(2) 24 hours Schick readings showed, in my experience that 3 per cent. proved later to be incorrect in a Council day school, where the children who had been tested were mostly under 10 years of age.

In an institution where the boys were all over 10 years of age, I found that 10 per cent. of the readings taken at 24 hours interval after the Schick test, were subsequently found to be incorrect.

The 7 days reading is, in my opinion, the most satisfactory.

(3) All Schick-positive reactors are not equally susceptible to diphtheria. The more brilliant the Schick-positive reaction the greater is the susceptibility to the disease.

(Two brothers at Carshalton slept in the same bed. One boy aet. 10 years, who had given a brilliant positive Schick reaction developed diphtheria while his brother aet. 7 years, who had given a slight Schick-positive reaction escaped.)

(4) In my series of Schick tests, 25 per cent. of those children who were known to have suffered from diphtheria prior to the Schick test showed a Schick-positive reaction.

(5) Pseudo reactions are extremely uncommon, in my experience, in the Council school children - most of the children tested were under 10 years of age.

Pseudo-negative reactions were not uncommon among Reformatory boys, whose average age is 16 years.

(6) That I have never seen any ill effects from the Schick test or the Schick control test.

(7) That it is possible to undertake Schick testing and toxoid-antitoxin injections at schools in the face of a sharp outbreak of diphtheria, provided that the parents of the children have been carefully informed that immunity may, in some cases, be slow in developing, and that a further injection, or injections, of T.A.T. may be necessary in certain cases, i.e. those still positive at the re-Schick test.

In no case can immunity certificates be issued until the final Schick test has proved negative.

(8) From the almost entire absence of pseudo reactions among children under 10 years of age it may be concluded that the control test is unnecessary in the younger children.

At the re-Schick the control test is essential.

(9) The Schick test is superfluous in pre-school age children, as we can assume that the great majority of these children are susceptible to diphtheria.

(10) The Reformatory school boys gave 52 per cent. Schick-negative reactors, a result which we might expect for the following reasons:-

(a) average age of the boys is 16 years.

(b) the boys come from poor homes in the overcrowded districts of London and other large towns.

Contrast the above with only 3 per cent. Schick-negative reactors at Wallington Council School, situated in a residential area, where the incidence of diphtheria is extremely low.

(11) It is of the utmost importance that the technique of the Schick test should be carried out with care, and some experience is necessary to do this accurately, and all tests, injections, and readings must be correctly recorded on each child's Schick card.

(12) Personally, I always destroy any material, whether Schick test toxin, or Schick Control, or

T.A.T., which may be left over after completing a Session's work. This is a precaution against any possible contamination from the air - a most unlikely contingency - but in this work it is wise to take no risks.

(13) Many of the schools Schick tested by me, showed an extraordinarily low basal immunity and, excluding the Reformatory school, about 93 per cent. (92.909) were Wchick-positive. In all cases a control test was made except for a few pre-school age children, who came into the scheme at their parents request. (N.B. The local maternity and child welfare centres are under the control of the local authority and not under the County Council).

(14) That the prophylactic employed by me in Surrey was entirely toxoid-antitoxin, and not toxin-antitoxin, and in view of the accidents reported with the latter in certain foreign countries, it is desirable that only toxoid-antitoxin, which is apparently a "safe" mixture, be used.

Toxoid-antitoxin should be suitably stored, preferably in an ice chest, and should not be used beyond the date stated on the label.

(15) Toxoid-antitoxin mixture, in my experience, has not caused any ill effects, except for a few trivial reactions in children over 12 years of age, and in some adults and, especially, when the full dose of 1 c.cm. was given to adults.

Control Schick tests are essential in persons over 10 years of age in order to eliminate pseudo-negative reactors, and to find pseudo-positive (i.e. combined) reactors; these latter are very rare, and they should be given smaller doses of T.A.T., as all pseudo reactors are much more liable to severe reactions are much more liable to severe reactions from T.A.T. injections.

(16) That when Schick work is carefully explained to parents, the latter are willing to have their children tested, and, if necessary, immunized.

That the process is judged by results, and although it is unfair, and premature, to make any definite statements about our results; yet the position appears somewhat suggestive and encouraging.

(Ballards School has remained free from the disease since the initial Schick work in 1926, and this institution was formerly greatly troubled by diphtheria.)

(17) That it is essential to secure the co-operation of the school teaching staff - in my experience the headmasters and other teachers gave us the greatest possible assistance.

(18) That it is desirable to secure the support of the local private medical practitioners when undertaking Schick work. This was generally achieved in our work, and I know that some of the parents signed consent forms after consulting the family doctor.

(19) Re-Schicking of all children who have completed the course of 3 c.cms. T.A.T., is undertaken at an interval of 6 months, or longer, after the last injection of toxoid-antitoxin mixture.

Should the re-Schick test prove to be positive, a further injection of T.A.T. is given, and the child is Schick tested again. No certificate of immunity to diphtheria can be issued until the final Schick is negative.

(20) Diphtheria of a very mild type occurred in three children who had completed 3 c.cms. T.A.T. injections, the intervals elapsing between the last injection and the onset of the disease being as follows:- (a) 5 days, (b) 4 weeks, (c) 4 weeks 5 days.

These three cases all made a rapid and uncomplicated recovery.

Diphtheria was also notified in 10 children before completing their course of 3 c.cms. T.A.T.; 4 of these cases occurred before any T.A.T. could be given, i.e. shortly after the Schick test.

4 of these cases had received 1 c.cm. T.A.T. only, and 2 cases had had 2 c.cms. T.A.T.

All of these ten cases occurred within 15 days of the Schick test, and they all recovered from the disease.

(21) That if a Schick-negative person be reported as suffering from diphtheria, a re-Schick test should, if possible, be performed immediately and before any antitoxic serum has been administered, and, further, 5 c.cms. of the patient's blood should be sent for laboratory estimation of the antitoxic content. Swabs from the nose and throat should be sent for bacteriological examination, and for virulence tests in those cases labelled as positive K.L.B.

(22) That it is inadvisable to give any adult patient an initial injection of 1 c.cm. T.A.T. It is better to give 0.1 c.cm. T.A.T. as a first

dose and proceed with doses of 0.5 c.cm. until the total course of 3 c.cms. has been given. Persons subject to migraine and asthma, and pseudo-combined reactors should not be given more than 0.05 c.cm. T.A.T. for the initial dose. It is generally possible to eliminate, or greatly minimize, unpleasant reactions by taking these precautions.

(23) That it is apparently possible to keep an Institution free from diphtheria when all the susceptibles have been artificially immunized by active immunization with T.A.T. injections to the point of becoming Schick-negative, and all the new comers are systematically dealt with.

Encouraging facts have been reported from Schick work among Fever Hospital Staffs, but time is required to come to definite conclusions on this point. That progress in the future may be made by increasing the antigenic efficiency of the prophylactic so that a considerable degree of immunity may be established, within a shorter space of time, and with fewer injections than are at present required.

In conclusion, the Schick test, which is of considerable importance is the diagnosis of

diphtheria, offers the best prospect of successfully controlling epidemics of diphtheria, and, especially so, in schools, hospitals, infant homes or other institutions. It must be once more emphasized that the clinical examination of the patient and contacts is the first, and most important step, and that all clinically suspicious cases should be tested with antitoxic serum immediately. Swabbing, nasal and faucial, of contacts is of considerable value and is, of course, carried out as a routine measure.

Schick-positive reactors should be injected with T.A.T., or if more immediate protection is indicated, prophylactic injections of antitoxin may be given. The difficulty in controlling the spread of the disease - a droplet infection - transmitted as it is in the great majority of cases by direct contact with cases and carriers, or through chains of carriers, has caused modern opinion to veer round to the view that the problem may be solved by the immunization of the susceptible individuals.

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